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CILIARY MOVEMENT AND CIRCULATION OF CEREBROSPINAL FLUID WITHIN BRAIN VENTRICLES IN LARVAL AND ADULT ANURANS

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In 1941 Wang and Lu reported that, when the living brain of a frog tadpole is examined under the microscope, dark particles are frequently seen darting to and fro in the brain ventricles. The movements of such dark particles offer an opportunity for studying the factors controlling the circulation of the cerebrospinal fluid. Therefore, it has been proposed to investigate *first*, the origin and fate of the dark particles, and *second*, the cause of their movements. Special attention has been paid to the second problem. Its solution has indicated that motion of the cilia on the ependymal cells covering the choroid plexus and the brain ventricles generates local currents in the cerebrospinal fluid and thereby plays a rôle in its circulation inside the ventricles. Observation has been extended to frogs, water toads and true toads, and similar results have been obtained. That the ependymal cilia play a part in the circulation of the cerebrospinal fluid hitherto has not been suspected. The results of our work here reported serve to call attention to this long neglected factor.

METHODS. Observations were made mainly on the tadpoles and adults of *Microhyla peulchra*. This species of anura was chosen for study because an abundant supply of its eggs and adults is found in the ponds near the laboratory of this Institute, and particularly because the body of its larva remains translucent almost up to the time of metamorphosis. Pigments in the skin of older larvae may obscure the contents of the brain case; but this is easily remedied by removing the skin before observation. For comparison, tadpoles and adults of *Rana limnocharis*, *Polypedates leucomystax* and one unidentified species of toad also were used.

Young larvae under observation were laid on a thin layer of moistened cotton wool on a glass slide; older ones were placed in a small water chamber built with

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wax on the slide. For studying the cause of the movements of black particles, blood of the larva diluted with amphibian saline solution was introduced into the brain ventricles. This was done by means of a micropipette operated by a simple home-made micromanipulator, in order to avoid the escape of the cerebrospinal fluid from the brain case. In one experiment, the ectodermal anlage of hypophysis was extirpated by sucking it out at the gill-slit stage.

RESULTS. A. *Observation on larvae. 1. The origin and fate of the particle.* Black particles in the brain ventricles of anuran larvae could be easily removed by first puncturing the skull over the caudal hindbrain, and then applying gentle pressure on the skull to force the particles to come out through the punctured hole. The removed particles were either dissected into small pieces or smeared on a slide. When these preparations were examined under the microscope at a magnification of 575 diameters, no cellular structures could be discerned. The particles simply consisted of black pigmentary granules. No sign of their regeneration was found up to 27 days after their removal.

For the purpose of tracing the course of development of the particles from their first appearance to their final disappearance, tadpoles of *Microhyla peulchra* were hatched and reared in the laboratory; and dissections were made either on freshly sacrificed larvae or on specimens fixed in Destin's solution from the neurula stage to several days after metamorphosis. Just after the neural tube was completely closed, its inner wall was found to be still covered by a layer of pigmented ectodermal cells, and no dark particles appeared. Shortly after this, dark particles made their first appearance, and they increased in number *pari passu* with the gradual disappearance of pigmented cells on the inner wall of the neural tube. With larvae approximately 4 mm. long, the pigmented cells on the inner wall almost all vanished; the number of dark particles reached its climax. Usually more than 20 particles were found in the third and fourth ventricle, some moving freely in the cerebrospinal fluid, while the rest still adhered to the ventricular wall. Gradually all particles became detached and free-moving bodies. Hereafter, they decreased in number but increased in size. Small particles seemed to coalesce with each other into a bigger one. Large particles usually lodged somewhere in the caudal part of the hindbrain; they were too heavy to be moved by the currents in the cerebrospinal fluid. One to three days after metamorphosis, large particles could still be found in the hindbrain ventricle. However, they were never encountered in the brain ventricles of adult forms of *Microhyla peulchra* and other species of anura examined.

This course of development was not affected either by hatching and rearing the larva in a dark room, or by removing the ectodermal anlage of the hypophysis at the gill-slit stage.

All these facts strongly suggested that the dark particles had their origin in the pigment of the ectodermal cells covering the ventricular wall. In several series of serial sections of the entire larva of *Rana limnocharis* prepared for another purpose, we found globules of dark pigment floating in the ventricle, adhering to the surface of the ependyma and lying between cells. These findings

gave support to our conclusion. Furthermore, an opportunity to test it was found in the eggs of *Polypedates leucomystax*.

The eggs of this species of tree frog are white in color. After hatching, the larvae also are devoid of pigmentation. A few pigmented cells appear on the dorsal surface of the body, when the tadpole grows to a body-length of 6 mm. Dissections were made at different developmental stages on tadpoles hatched and reared in the laboratory. In the brain ventricles of larvae less than 6 mm. long, no dark particles were ever encountered. They first appeared after the dorsal surface of the body became scantly pigmented. But their number (3 or 4) was 5 to 7 times less than the maximum found in the larvae of the Microhyla. At a body-length of approximately 12 mm., the 3 or 4 particles were reduced to 1 or 2. Dark particles completely disappeared in tadpoles longer than 14 mm.

The data presented in the preceding paragraphs justify the following conclusion: the dark particles found in the brain ventricles of anuran tadpoles have their origin in the pigment of the epithelial cells covering the inner wall of the neural tube, and are gradually absorbed after the small particles have coalesced with each other into bigger ones.

2. *The cause of its movements.* When a living Microhyla tadpole 5 mm. long was examined under the microscope, several small dark particles were usually seen moving actively in the third and fourth ventricles of the brain. The motion of each particle consisted of both rotation and translation in a straight or curvilinear path. It darted to and fro in the ventricles, or circled around as if in a whirlpool. But both the rhythm and the path of its movements were highly irregular. With larger older larvae, 1 or 2 moving particles were observed. As the size of the particle reached the upper limit (approximately 2.5×10^{-4} mm., as determined by measurements of removed particles), it ceased to move, and was usually anchored somewhere in the caudal half of the hindbrain ventricle. Medium-sized particles frequently were caught in the corners of the ventricles during their motion.

The movements of dark particles were influenced neither by the exclusion of circulation nor by changes in the position of the body in space. Exclusion of circulation by decapitation rendered bloodless the vessels in the head and thereby gave a clearer field for observation. No change in the motion of dark particles was noticed, except that their movements progressively grew weaker as the preparation slowly deteriorated. With the microscope in the horizontal position, a small tadpole placed on a thin layer of cotton wool on a slide could be rotated on the dorsiventral axis of its body into any position in a vertical plane. Such change of position in space was found to have no observable effect on the motion of particles in the brain ventricles.

A convenient means for investigating the cause of particle movements was provided by the following accidental observation. In the attempt at removing the particles, some blood vessels of the skull were always severed, when the skull was punctured. Red blood corpuscles escaped into the ventricles, and showed similar movements to those of the dark particles. Then, blood from large tad-

poles or adults diluted with amphibian saline solution was deliberately injected into the fourth ventricle of the larva under observation; and the injected red cells moved in a highly similar manner as the particles originally in the ventricle. This gave us a method for direct inspection of the cause of particle movements.

When a few drops of diluted blood were introduced into the cerebrospinal fluid in the exposed fourth ventricle, the added red blood corpuscles showed the same movements as in the intact ventricle. As soon as the cerebrospinal fluid was drained away, the red cells rested motionless on the ependyma. This piece of evidence clearly indicates that the movements of the particles in the ventricles are due to local currents in the cerebrospinal fluid.

In order to demonstrate how such local currents are generated, pieces of the ventricular wall were removed and examined under the microscope. When a piece of the ventricular wall placed in a bath of diluted blood was observed under the low power (magnification: *circa* 40 diameters), red cells were seen moving near the ependymal surface. These movements were similar to those of red blood corpuscles near the ciliated surface of a piece of esophagus removed from frog and examined in the same manner. Since it is known that the ependymal epithelium also possesses cilia on its free surface, it seemed reasonable to conclude that the local currents near the ependyma were due to ciliary movements. However, the attempt at direct inspection of ciliary movement on the ependyma under high power (× 575 diameters) entirely failed. Another trial to observe the movements of ependymal cilia in the reflected light with the 'ultropaque' microscope was also unsuccessful. The mass of nervous tissue adhering to the ependyma interfered with observation in both transmitted and reflected light; and no method was found to free the ependyma from nervous tissue without damaging the former.

The ependyma on the roof of the fourth ventricle also has cilia, and forms a thin sheet of cells suitable for microscopic examination. Placing a piece of the roof membrane stained with methylene blue and moistened with diluted blood under high power (× 575 diameters), we could observe the red blood corpuscles following the movements of the ependymal cilia. This was most clearly observed when the preparation was dying and the ciliary movements became slow.

Hence, the cause of the movements of dark particles in the brain ventricles is found in the local currents generated by the motion of ependymal cilia in the cerebrospinal fluid.

B. *Observations on adults.* Two series of observations were made on the adults of *Microhyla pculchra*, *Rana limnocharis*, *Polypedates leucomystax* and one unidentified species of *Bufo*. In one series, the movements of added red blood corpuscles in the exposed third and fourth ventricle of the removed brain were observed, whereas in the other we watched under the microscope at a magnification of 575 diameters the motion of the cilia on a piece of the roof of the fourth ventricle removed from the brain and stained with methylene blue. The results of both series were similar to the findings obtained with identical methods on the larva.

Discussion. The results presented in the preceding section definitely

demonstrate that movements of the cilia on the ependymal lining of ventricles play a rôle in the circulation of the cerebrospinal fluid inside the ventricles, at least in amphibians. When we take into consideration the enormous number of these cilia, we must concede that their rôle in the motion of the cerebrospinal fluid is an important one. This observation appears to be new. Cilia have been known to exist on the ependymal epithelium since the time of Purkinje. Yet both anatomists and physiologists seem not to have suspected that these cilia have any relation to the motion of the cerebrospinal fluid, as shown by the latest reviews of the literature on the structure of ependyma (Wislocki, 1928; Agduhr, 1932) and on the circulation of the cerebrospinal fluid (Abderhalden, 1927; Plaut, 1927).

According to Studnicka (1900), cilia are present on the ependyma with great regularity in lower vertebrates and during fetal life in mammalia. But, in adult mammals, they are present only in restricted areas. These facts suggest that ciliary movement may not play any part in the circulation of the cerebrospinal fluid in mammals. However, records thus far published by different investigators of brain pulses taken from trephine holes on the skull of different mammals including man throw some doubts on this suggestion. All records register many highly irregular oscillations, in addition to the waves corresponding either to the heart beat or to the respiratory rhythm. Such irregular oscillations have not yet been explained, and most probably are caused by the synchronized movements of a great many ependymal cilia. Furthermore, observations on rats also are against the above-mentioned suggestion. In our experiments we added a few drops of diluted frog's blood to the mammalian saline solution in the exposed third or fourth ventricle of a removed brain, and found the frog's red blood corpuscles moving in the same manner as those introduced into the ventricle of an anuran. The whirling about of the frog's red blood cells in the rat's ventricle certainly indicates that the cilia on the ependyma are in constant motion, although we have not succeeded in observing directly the movements of the cilia on a piece of the roof of the fourth ventricle removed from the rat. Thus, ciliary movement most probably plays the same rôle in the circulation of the cerebrospinal fluid in mammalia just as in amphibia.

SUMMARY

Observations are made on the movements of some dark particles in the brain ventricles of living anuran larvae. The dark particles have their origin in the pigmented cells of the ependymal covering over the inner wall of neural tube. They persist throughout the larval life; and they are absent in the adults. The movements of dark particles are due to the local currents in the cerebrospinal fluid generated by the motion of the cilia on epethelial cells lining the ventricles.

Observations on frogs and toads show that movements of the cilia on the ependyma also produce local currents in the cerebrospinal fluid.

The significance of ciliary movements as a hitherto neglected factor in the circulation of the cerebrospinal fluid within the brain ventricles is discussed.

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URINE DILUTION AND CONCENTRATION TESTS IN ADRENALECTOMIZED DOGS

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Evidence has accumulated which indicates that during advanced adrenal insufficiency the ability of the kidney to perform its normal functions is diminished. There is a reduction of the elimination of urea, so that this substance accumulates in the blood (1). Likewise, the renal excretion of potassium is reduced and its quantity in the blood is increased (2, 3). At the same time the power of the kidney to retain sodium and chloride is diminished, so that these substances are lost from the body (3, 4, 5). The present investigation was undertaken with a twofold aim: first, to determine the power of the kidney to do its work in a general rather than a specific manner in the absence of adrenal cortical hormone, but with the animal in good condition; second, under similar circumstances to determine the ability of the kidney to excrete excess chloride.

PROCEDURE AND METHODS. To achieve the purposes of this research, urine dilution and concentration tests and a renal chloride concentration test were carried out on dogs before and after adrenalectomy. After adrenalectomy these tests were performed when the animals were maintained in good condition as described subsequently without cortical hormone. Healthy dogs weighing from 12 to 15 kgm. were used. The adrenal glands were removed with the usual surgical technic under ether anesthesia at separate operations. An interval of three weeks or more was allowed between operations. As a routine all animals were dewormed with tetrachlorethylene prior to removal of the second adrenal.

It was important in this study to maintain the animals on the same diet during the kidney function tests both before and after adrenalectomy so that alterations in this factor would not complicate the tests. A high sodium-low potassium diet was used. It was composed of casein, 12 per cent; whole wheat flour, 15 per cent; sucrose, 10 per cent; lard, 10 per cent; butter, 5 per cent; yeast, 2 per cent; cod liver oil, 1 per cent; sodium chloride, 2.5 per cent; sodium citrate 1.5 per cent; bone ash, 1 per cent; and water, 40 per cent. The ingredients were thoroughly mixed and then baked in a slow oven to the consistency of soft cake. The animals were fed 300 to 350 grams of this diet per day and on this quantity they maintained their body weight or gained. At times, particularly immediately after adrenalectomy or when early signs of insufficiency were present, it was necessary to feed the diet by hand. As a routine, tablets of yeast concentrate

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were added to the cooked diet rather than cooked with the diet. In order to increase the sodium intake further, sodium chloride was added to the drinking water in quantities sufficient to make a 0.5 per cent solution. The total sodium intake was about 6.0 grams per day while the potassium intake was less than 300 mgm. per day.

The intact animals were placed on the high sodium-low potassium diet for at least three days before a series of control tests was run. The urine dilution and concentration tests and the chloride concentration test were performed in series with one day rest between the dilution and concentration tests. The urine chloride concentration test was carried out immediately after either the dilution or concentration test, before any food or water had been given to the dog. Tests were performed on some animals after one of the adrenal glands had been removed. No difference was found between these and tests on intact animals.

After the control tests had been completed satisfactorily, the second adrenal was removed. After the operation the high sodium diet was supplemented by the intravenous administration of hypertonic solutions of sodium chloride and glucose and by the subcutaneous injection of cortical extract or desoxycorticosterone acetate in sesame oil. Injections of hypertonic solutions of salt and glucose were discontinued after two or three days. Treatment with cortical extract or desoxycorticosterone acetate was stopped eight to ten days after operation when the wound was well healed and the animal in good condition. A period of seven to ten days was allowed to elapse before any renal function tests were performed to insure that all of the cortical substitute had been destroyed or excreted.

Urine dilution test. After a twenty-four hour fast, the animals were catheterized and then given 300 cc. of water by stomach tube. Catheterization was repeated at hourly intervals for four to seven hours. The volume, specific gravity, and chloride concentration of the urine were determined for each hour sample.

Urine concentration test. The concentration test was started in the morning by catheterization of the animal and removal of water from the cage. The standard daily ration was then given and from this time until the end of the test the animal did not receive any further food or water. As a routine animals were catheterized at least morning and evening. The volume, specific gravity, and chloride concentration were determined for each sample of urine.

Urine chloride concentration test. In an attempt to determine the maximal ability of the kidney to concentrate chloride in the urine a test was developed in which a hypertonic solution of sodium chloride was given by vein and the chloride concentration in the urine was followed at definite intervals. This test was carried out immediately after either the urine concentration or urine dilution test. The procedure followed was to inject 350 cc. of 4.5 per cent solution of sodium chloride into the saphenous vein at a constant rate of 10 cc. per minute. Blood samples were taken prior to injection, just before the end of the injection, and thirty minutes after the injection was completed. A catheter was introduced into the bladder and left in place for the duration of the test. The bladder was drained before the injection was started and at ten minute intervals until the

diuresis became less than about 1 cc. per minute when the collection time was lengthened to twenty to thirty minutes to allow larger samples for analysis. As a rule the ten minute samples were obtained for about two hours and sampling at the longer interval continued for a further hour.

The specific gravity of the urine was determined by weighing samples delivered from a 1 cc. syringe-pipet of the Krogh-Keys type. To offset the loss of weight by evaporation during weighing the urine was delivered into a 25 cc. Erlenmeyer flask counterbalanced by a similar flask containing water. All samples were weighed to 0.1 mgm. on an analytical balance. Single and triple deliveries from the syringe-pipet were used to determine the weight of the standard urine volume. Experiments showed that the reproducibility of results of triple deliveries was slightly more accurate than for single deliveries; therefore whenever the urine samples were sufficiently large, triple deliveries were used. The standard deviation determined by triple delivery was 0.5 mgm. Any figures considered significant in this work were many times as large as this figure. Corrections for changes in weight due to variations in the temperature at the time of weighing were made by use of standard tables of water density.

Throughout this study all animals were kept in cages suitable for urine collection. During the various tests all samples of urine were obtained by catheterization. To insure complete emptying of the bladder, the urine was withdrawn through the catheter under gentle suction with a 50 cc. syringe and light pressure was applied to the lower part of the abdomen. Blood samples were taken from the saphenous or jugular vein into oiled syringes containing heparin as an anticoagulant. The amount of urea in the blood was used as one indication of the condition of the adrenalectomized animals. It was estimated by the urease aeration method of Van Slyke and Cullen (6). Urine chloride estimations were done by the Van Slyke modification of the Volhard-Harvey titration method. The Van Slyke and Sendroy application of the open Carius method was used to determine the plasma chloride. Blood cell volume ratios were estimated by centrifuging 10 cc. samples of blood in recalibrated graduated centrifuge tubes at 2800 r.p.m. for thirty minutes.

All of the animals used in this study eventually died with the typical signs of adrenal insufficiency and at necropsy no adrenal tissue was found.

Results. I. *Dilution tests.* Seventeen dilution tests were carried out on eight healthy intact dogs maintained on the high sodium-low potassium diet for at least three days before performance of the test. The results of these control tests were compared with those obtained in fourteen tests on five adrenalectomized animals maintained on the same regimen. Before the water for the test was given, the bladder was emptied. The specific gravity of this initial urine was variable, ranging from 1.039 to 1.007 in the intact animals and from 1.034 to 1.011 in the adrenalectomized. Despite this wide variation of the initial specific gravity values, the low levels reached during the first and second hour after administration of the water were quite constant in both the normal and the adrenalectomized animals (table 1). During the third hour the specific gravity rose sharply in both the normal and adrenalectomized dogs.

When the values of the specific gravity of the urine are charted against time, a

curve for the dilution test is obtained. In each animal the mean curve of all the tests performed before adrenalectomy was compared with the mean curve of all the tests performed after adrenalectomy. In each animal the mean curve fell to a lower level before adrenalectomy than after adrenalectomy.

Comparison of the various *mean* specific gravity values in the normal and adrenalectomized animals suggested some reduction of the ability of the adrenal-

TABLE 1

Specific gravity of urine during dilution tests in normal and adrenalectomized dogs

PERIOD	NUMBER		SPECIFIC GRAVITY OF URINE			
	Tests	Dogs	Maximum	Minimum	Mean	Standard deviation
Normal animals						
1st hour	17	8	1.0110	1.0005	1.0042	0.0032
2nd hour	17	8	1.0063	1.0002	1.0033	0.0018
3rd hour	13	7	1.0134	1.0010	1.0071	0.0046
4th hour	11	6	1.0209	1.0050	1.0136	0.0046
Adrenalectomized animals						
1st hour	14	5	1.0303	1.0057	1.0142	0.0072
2nd hour	13	5	1.0211	1.0017	1.0084	0.0064
3rd hour	14	5	1.0185	1.0037	1.0096	0.0047
4th hour	14	5	1.0215	1.0043	1.0110	0.0052

TABLE 2

Urine dilution tests during adrenal insufficiency

DOG	URINE SAMPLE		CLINICAL CONDITION
	Time taken during test	Specific gravity	
	hours		
H-1	2	1.022	Anorexia, vomiting, progressive weakness
	2	1.011	Anorexia, vomiting, listlessness
F	2	1.015	Anorexia, plasma K 31.3 mgm. per 100 cc.
	3	1.013	24 mgm. urea nitrogen per 100 cc. blood
S	2	1.021	Anorexia, vomiting, progressive weakness
	3	1.019	

ectomized dog to produce a dilute urine. Further study showed that such a conclusion was unjustified. The results of the tests on the adrenalectomized animals were more variable than those obtained from the intact animals. It was found that the ability of the adrenalectomized animals to dilute the urine was dependent on the success with which they were maintained on the high sodium-low potassium diet. When maintenance was inadequate, the adrenalectomized animals were unable to dilute the urine below a specific gravity of 1.010 (table 2).

On the other hand, when the adrenalectomized animal was in the best clinical condition the results of the dilution test were practically the same as those obtained in the intact animal.

To emphasize this finding further, the best performance of each dog before adrenalectomy was compared with the most satisfactory performance obtained after adrenalectomy (fig. 1). In each animal the greatest dilution of the urine occurred before adrenalectomy but the differences in the lowest specific gravity values obtained before and after adrenalectomy (0.0002, 0.0004, 0.0009, 0.0018 and 0.0050 respectively) were, with the exception of the last instance, so slight that no special significance has been attached to them. Three of the differences are within the range of error of the specific gravity estimation. The experiments indicate that the ability of the kidney of the adrenalectomized dog to dilute the urine is not impaired when the animal is maintained in good clinical condition on a high sodium-low potassium diet without cortical hormone. When signs of

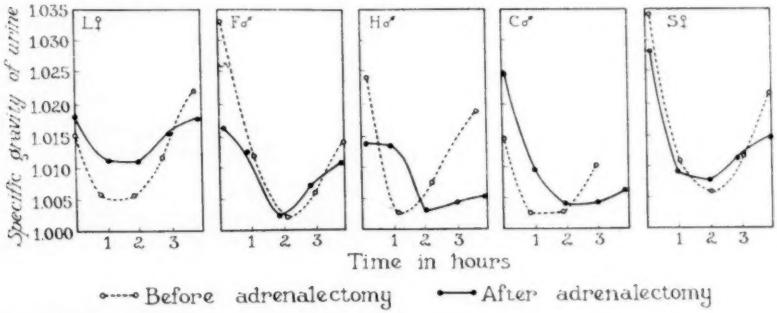

Fig. 1. Specific gravity of urine during urine dilution tests, before and after adrenalectomy in dogs L, F, H, C and S maintained on a high sodium-low potassium diet without cortical hormone.

adrenal insufficiency supervene there is a rapid deterioration in the urine diluting power of the kidney.

II. *Concentration tests.* Early in the study it became evident that the degree of concentration reached in the urine varied with the environmental conditions. As might be expected, during hot summer weather the concentration of the urine was considerably greater than during the remainder of the year when moderate temperatures prevailed in the animal quarters. The concentration tests therefore have been separated into two groups, the moderate temperature tests and the hot weather tests.

During the moderate temperature tests the specific gravity of the urine of the intact animals maintained on the high sodium-low potassium diet usually exceeded 1.035 forty hours after food and water had been taken from the cages (fig. 2). Fifteen tests of more than twenty hours' duration were carried out on the intact animals. In twelve of these a specific gravity of more than 1.035 was observed at some time during the test. In the remaining three a specific gravity

of more than 1.030 was obtained. With the intact animal, a concentration of more than 1.032 was attained in every test which was continued for more than thirty hours. Only two tests at moderate temperatures were carried out on adrenalectomized animals. In these, the specific gravity of the urine did not exceed 1.028 during the first seventy hours of the test and the highest concentration reached after seventy hours was 1.032. The results suggested some reduction in the power of the kidney of the adrenalectomized dog to secrete a concentrated urine. Definite differences, however, were encountered in the hot weather tests and for this reason the tests at moderate temperatures were discontinued.

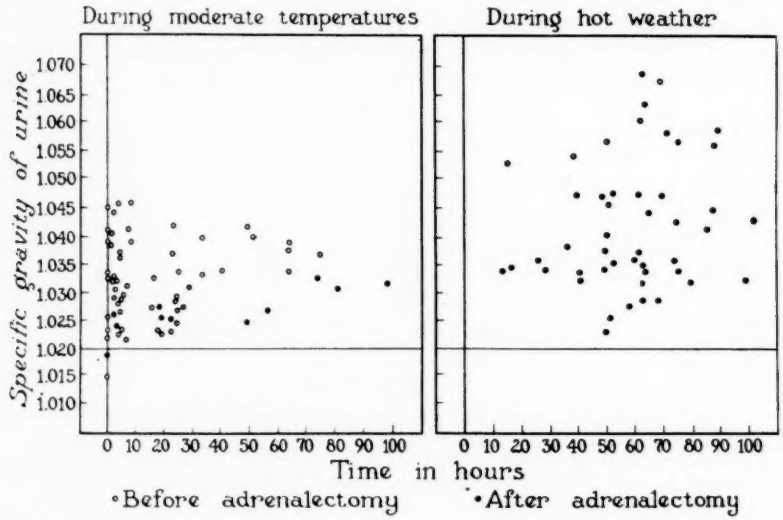

Fig. 2. Specific gravity of the urine of intact and adrenalectomized dogs during urine concentration tests in hot and moderate weather. All animals maintained on high sodium-low potassium diet without cortical hormone.

The concentration tests during high environmental temperatures were controlled by studies on three intact dogs. All achieved urine concentrations of more than specific gravity 1.058 (fig. 2). The highest specific gravity was 1.066 and the average of the series was 1.062. In contrast, the highest urine specific gravity reached in seven tests on five adrenalectomized animals during the same hot weather period was 1.046 (fig. 2).

One of the adrenalectomized female dogs had been maintained in excellent condition on the high sodium-low potassium diet alone for more than thirty days before tests were run. This animal then had the appearance and vigor of a normal intact dog. Its concentration tests were carried out during the hottest summer weather, when the temperature reached 100°F. or more for several consecutive days. The maximal specific gravity values reached during these

tests were 1.041 to 1.046. These values are 0.010 to 0.015 less than the maximal concentration obtained in intact dogs.

During the course of these tests it was noticed that the ability of the adrenalectomized animals to elaborate a concentrated urine could be correlated with the general condition of the animal. All the results reported in the previous paragraphs were obtained on animals in good clinical condition. When signs of insufficiency were present some reduction of the concentrating power was evident.

The results of the concentration tests indicate that the power of adrenalectomized dogs maintained on a high sodium-low potassium diet without cortical hormone to concentrate urine is reduced even when these animals are in excellent general condition.

III. *Chloride concentration tests.* There is adequate evidence of impairment of the ability of the kidney of adrenalectomized animals to produce a urine of low chloride content, but little is known of the upper limit of chloride concentrating power in these animals. The chloride concentration test was devised to determine this maximal chloride concentrating power in adrenalectomized animals maintained in good health, without cortical hormone, on the high sodium-low potassium diet.

In performance of the test, after the bladder had been emptied a 4.5 per cent solution of sodium chloride was injected intravenously at the rate of 10 cc. per minute for thirty-five minutes. In the preliminary tests on intact dogs the hypertonic solution was injected under two contrasting states of body hydration. This was accomplished by performing the test immediately after either the urine dilution test or the urine concentration test. When the chloride concentration test followed the dilution test, excess water had been administered only a few hours previously but when the chloride concentration test followed the urine concentration test, no water had been taken for at least fifty hours. It was soon found, as might be anticipated, that greater concentrations of chloride in the urine were obtained when the hypertonic saline solution was injected immediately after a concentration test than when the injection followed a dilution test. As a routine, therefore, the hypertonic salt solution was given after a concentration test.

Urine volume production. About twenty minutes after the injection had been started, the rate of urine production increased sharply. The maximal rate usually was reached during the first hour, after which urine production gradually declined (fig. 3). In the intact animal when the salt was given after a dilution test, the diuresis was more prolonged and the maximal rate of production of urine often higher than when the injection followed a concentration test (fig. 3). The total volume of urine formed was also greater when the salt was given immediately after a dilution test than when it was given after a concentration test.

When the salt injection followed a concentration test, the maximal rate of production of urine ranged from 4.6 to 8.7 cc. per minute in the intact animals and from 2.7 to 5.5 cc. per minute in the adrenalectomized animals. The decline of the diuresis was somewhat more rapid in the intact than in the adrenalecto-

mized dogs, so that by the end of the test there was not much difference between the total volumes of urine eliminated by the intact and adrenalectomized animals (fig. 3*a*).

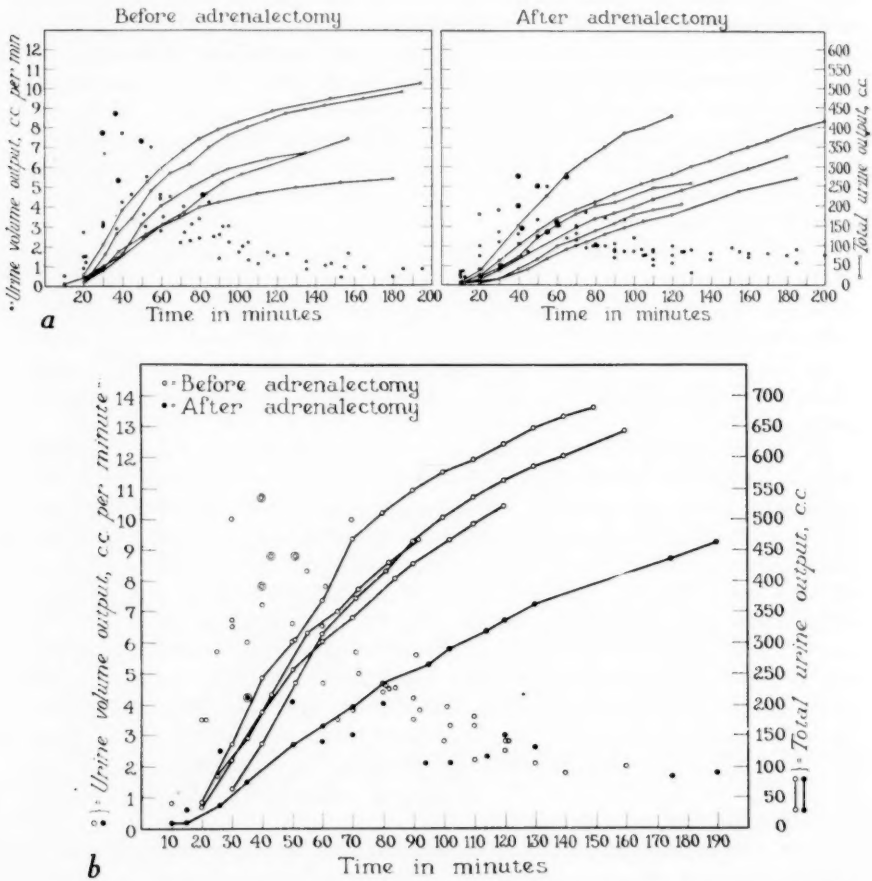

Fig. 3. Rate of production of urine and total volume of urine produced during chloride concentration tests in intact and adrenalectomized dogs maintained on high sodium-low potassium diet without cortical hormone. *a*, When chloride concentration tests were done immediately after urine concentration tests; *b*, when chloride concentration tests were done immediately after urine dilution tests. Double circles indicate the maximal rate of urine production in individual tests.

In the adrenalectomized dogs only one of the chloride concentration tests was done immediately after a dilution test. The rate of production of urine and the total volume of urine produced during this single test were distinctly less than those observed during the four control tests carried out under similar circumstances (fig. 3*b*).

In general, there was a definite tendency for the rate of production of urine in the adrenalectomized animals to rise slowly to a maximum and remain fixed at this level throughout the test. As a rule this rather fixed rate of production of urine in the adrenalectomized dogs was less than the maximal rate of production of urine observed in intact animals.

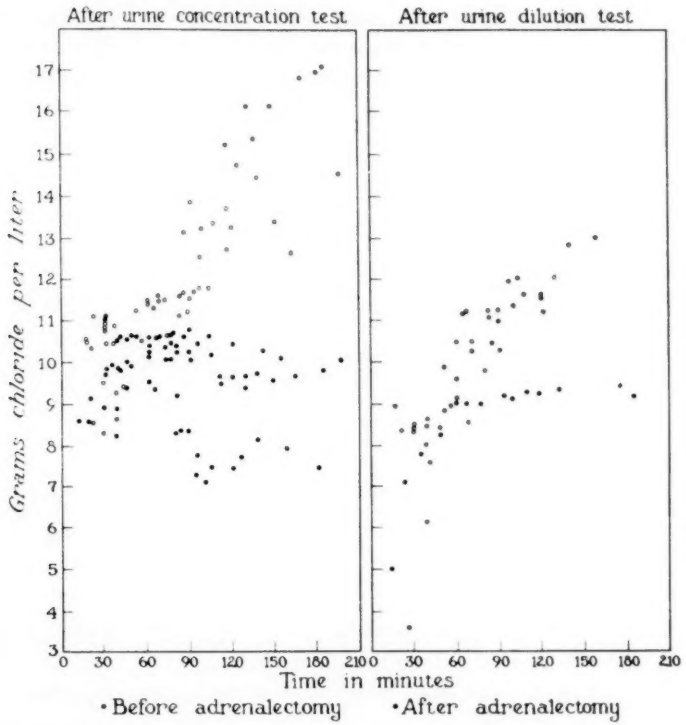

Fig. 4. Chloride concentrations in the urine of intact and adrenalectomized dogs during chloride concentration tests which were done immediately after urine concentration tests or urine dilution tests. Animals maintained on high sodium-low potassium diet without cortical hormone.

Urine chloride concentration. During the period of diuresis, the chloride content of the urine varied over approximately the same range of 6 to 10 grams per liter in both the intact and adrenalectomized dogs (fig. 4). During the later minutes of the test, after the diuresis had subsided, there was a marked difference between the chloride concentrations attained by the intact animals and those reached by the adrenalectomized animals. In the intact dogs after the period of diuresis, the chloride concentration rose to values varying from 13.2 to 17.9 grams of chloride per liter when the salt was given after a concentration test and from 10.7 to 13.0 grams when the salt was given after a dilution test. In

contrast to the intact animals the urine chloride concentration of the adrenalectomized animals did not rise after cessation of the diuresis but remained relatively fixed at levels far below those observed in the intact animals (fig. 4). Throughout the tests the highest urine chloride concentration found in an adrenalectomized animal was 11.6 grams per liter. In all other instances the maximal chloride concentration attained by an adrenalectomized animal was 11.0 grams per liter or less. The difference between the intact and adrenalectomized animal was most noticeable when the tests carried out before and after adrenalectomy were compared in the same dog (fig. 5). The experiments indi-

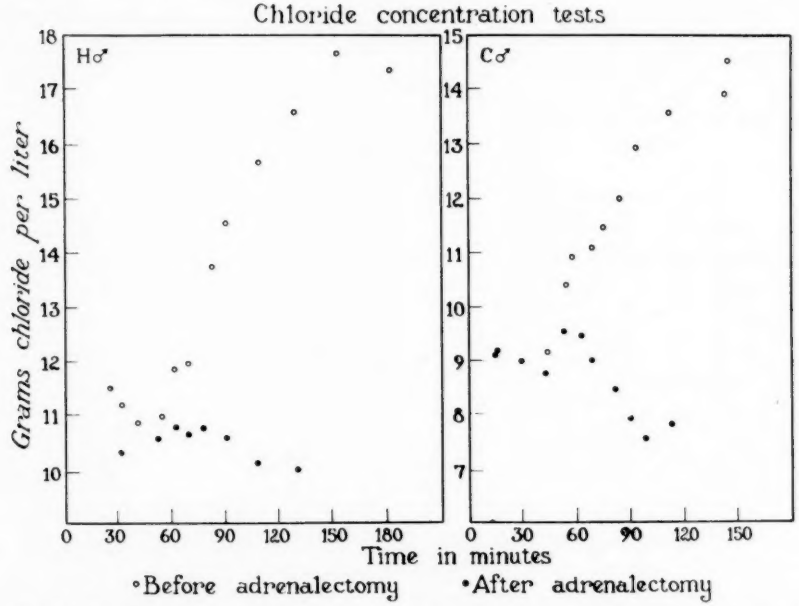

Fig. 5. Concentrations of chloride in the urine of two dogs, H and C, during chloride concentration tests before and after adrenalectomy. Animals maintained on high sodium-low potassium diet without cortical hormone.

cated that in the adrenalectomized animal, even when well maintained on a high sodium-low potassium diet without cortical hormone, the kidney had lost its ability to concentrate chloride in the urine above a ceiling of approximately 11 grams of chloride per liter of urine.

Urine chloride output. The changes in the chloride output per minute followed roughly the same pattern as the changes in rate of production of urine. In all instances, the most rapid elimination of chloride occurred during the period of diuresis, after which it gradually declined as production of urine was reduced (fig. 6). In the intact animals the rapid rate of elimination of chloride was continued for a longer period when the chloride was given after a dilution test than when administered after a concentration test. As a result, in the intact

animals the total chloride eliminated during the test was generally greater when the salt was injected after a dilution test (fig. 6). In the adrenalectomized dogs the rate of chloride elimination was generally slower during the first two hours

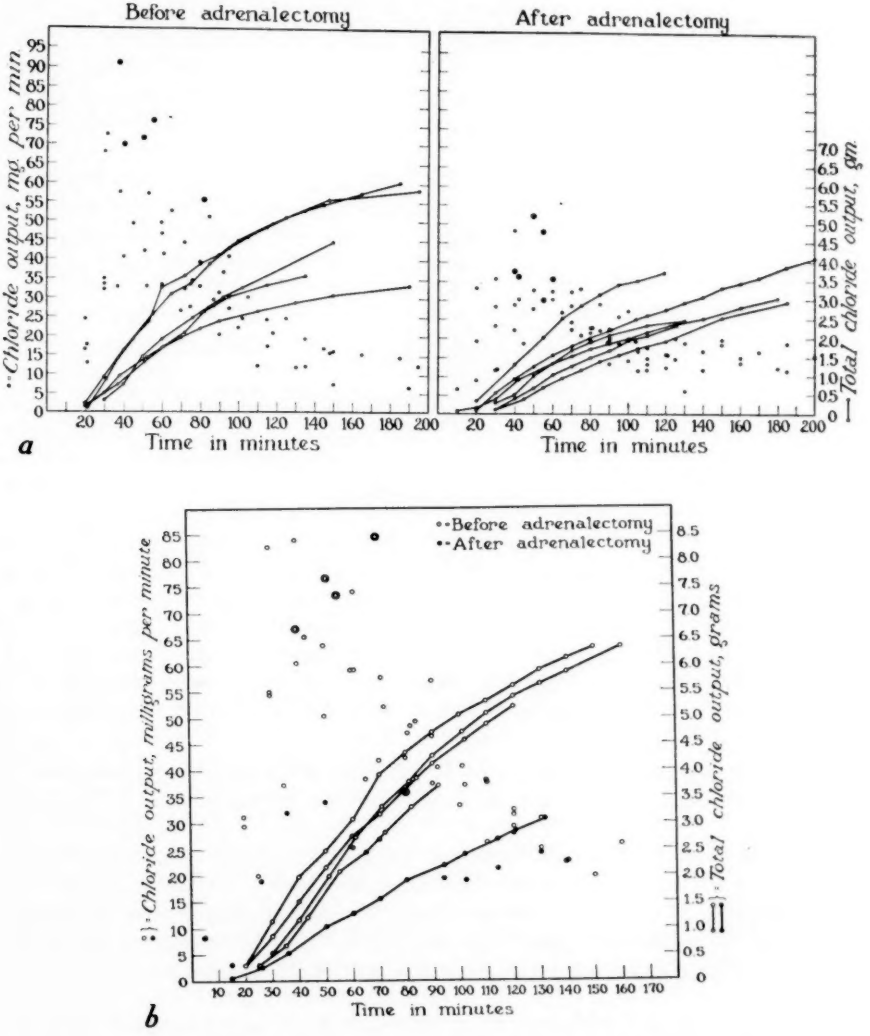

Fig. 6. Rate of chloride output and total chloride output in the urine during chloride concentration tests in dogs before and after adrenalectomy; *a*, when chloride concentration test was done immediately after a urine concentration test, and *b*, when chloride concentration test was done immediately after a urine dilution test. All animals maintained on high sodium-low potassium diet without cortical hormone. Double circles indicate maximal rate of chloride output in each test.

of the test, than in the intact animals. The maximal chloride output per minute was always less in the adrenalectomized than in the intact animals. Throughout all the tests the rate of elimination of chloride in the adrenalectomized dogs was more or less fixed in comparison with that of the intact animals (fig. 6). The total quantity of chloride eliminated during the tests was somewhat greater in the intact than in the adrenalectomized animals although the differences were not striking. The greatest amount of chloride was excreted when the salt injection was preceded by the administration of water (fig. 6*b*). The total quantity of chloride eliminated during the two to three hours of the test was always less than the quantity injected.

It seemed possible that the differences between the normal and the adrenalectomized animal might be due to differences in the concentration of chloride in the plasma delivered to the kidney. Blood samples were drawn thirty and sixty minutes after the beginning of the injection and the cell volume and plasma

TABLE 3

Red cell and plasma chloride concentrations during the chloride concentration test

DOG	PER CENT RED CELLS			MGM. CHLORIDE PER 100 CC. PLASMA		
	Before test	Minutes during test		Before test	Minutes during test	
		30	60		30	60
Before adrenalectomy						
H-2	54.6	38.4	42.8	392	503	441
S	46.0	31.8	37.8	419	481	470
After adrenalectomy						
L	52.0	42.3	42.1	391	485	473
H-2	57.7	34.8	41.0	390	495	456
L	46.8	42.9	39.0	391	475	483
L	49.0	32.5	39.0	396	465	398

chloride concentration determined. No marked differences were noticed between the values obtained for the intact and the adrenalectomized animals (table 3).

The data indicated that the kidney of the adrenalectomized animal had lost some of its flexibility despite the administration of adequate quantities of sodium chloride. In these experiments the maximal urine chloride concentrating power of the adrenalectomized dog, maintained in good health on the high sodium-low potassium diet without cortical hormone, was less than that of the intact dog.

COMMENT. In this study an attempt has been made to determine the extent to which the kidney, deprived of adrenal cortical hormone but supplied with sodium chloride, can change the concentration of solids in the urine. The specific gravity of the urine was used because it gives some measure of the ratio of water to dissolved substances. When subjected to a dilution test the kidney of the adrenalectomized animal was found to be able to elaborate as dilute, or

nearly as dilute, a urine as the intact animal. In the presence of sufficient sodium chloride, lack of cortical hormone did not limit seriously the ability of the tubule to reabsorb the crystalloids and leave the water of the glomerular filtrate.

In the concentration test some measure was obtained of the ability of the tubule to reabsorb water selectively and thus to allow accumulation of dissolved substances in the urine. During the hot weather tests the tubule without cortical hormone was unable to carry out this differential reabsorption of water sufficiently to elevate the specific gravity to the values obtained in the normal dogs. It seems unlikely that there was sufficient difference in the pattern of solids excreted in the urine of the intact and adrenalectomized dogs to account for the differences in the specific gravities of the urine samples. Apparently the high sodium-low potassium diet did not restore tubular function completely. It should be pointed out, however, that in this test the difference between the performance of the intact and adrenalectomized animals was not great. On the basis of general clinical experience with the urine concentration test the adrenalectomized animals on the high sodium-low potassium diet showed a degree of urine concentrating power usually considered sufficient for ordinary body needs. Despite this rather satisfactory over-all performance by the kidney the experiments definitely point to a reduction in the power of the tubule to reabsorb water selectively.

This was emphasized again in the chloride concentration tests in which the tubule without cortical hormone was unable to reabsorb water differentially sufficiently to raise the concentration of chloride in the urine to more than approximately 11 grams per liter. It may be considered that in these latter experiments the tubule was unable to refuse selectively to reabsorb chloride rather than unable to reabsorb sufficient water selectively. No matter which view is taken, the data indicate some reduction of the tubular function of the kidney supplied with ample sodium chloride but deprived of cortical hormone.

During the dilution tests the rate of formation of urine was, as a rule, somewhat slower in the adrenalectomized animals than in the intact animals. The water of the test was given by mouth and the slower rate of elimination in the adrenalectomized animals was possibly partly due to a reduced rate of absorption of the water from the gastro-intestinal tract (7). In the light of the findings of Harrison and Darrow (3) the possibility of a maximal rate of glomerular filtration somewhat less than that of the normal animal may also be considered as a possible cause of the slower rate of elimination in the adrenalectomized animals. A smaller volume of glomerular filtrate with a resulting slower passage through the tubules may have assisted the kidney of the adrenalectomized animals to produce an extremely dilute urine. No significant difference was noticed in the concentration of chloride in the urine during the dilution tests of the intact and adrenalectomized animals. Both groups of animals had been receiving large quantities of chloride in the high sodium-low potassium diet up to the time of giving the test. It seems probable that excess chloride was present in the body and that for this reason no difference in chloride conservation by the kidney was noticed during the test.

Willson and Sunderman (8) observed only a moderate increase of chloride

and sodium in the urine of a patient suffering from Addison's disease during a period of water deprivation. This observation suggested a diminution of the ability of their patient to concentrate sodium and chloride in urine. The present study has shown quite definitely that the maximal urine chloride concentrating power of adrenalectomized dogs, maintained in good health on the high sodium-low potassium diet without cortical hormone, is less than that of the intact dog.

Maintenance of mineral balance on an average diet necessitates the elimination of potassium and at times the conservation of both sodium and chloride. To accomplish this the kidney is required to concentrate potassium and if necessary dilute sodium and chloride in the urine. Retention of potassium and loss of sodium and chloride in the animal suffering from adrenal insufficiency have been explained as being due to a specific inability of the kidney to meet these requirements by failing to produce the necessary concentration gradients between plasma and urine (3). In this study it has been shown that the renal dysfunction of adrenalectomized animals is not limited to failure to concentrate potassium and to dilute sodium and chloride in the urine but also includes a reduction in ability to concentrate chloride in the urine. For it was found during the chloride concentration tests that the adrenalectomized animal failed to concentrate chloride to the extent of the normal and as a result there was actually an abnormal retention of this ion. Thus when high concentrations of chloride are required in the urine, the kidney of the adrenalectomized animal behaves toward chloride as it has been shown to behave toward potassium when high concentrations of potassium are required in the urine.

SUMMARY AND CONCLUSIONS

The kidney function of adrenalectomized dogs maintained in good condition on a high sodium-low potassium diet without cortical hormone was tested by a urine dilution test, a urine concentration test and a chloride concentration test and the results were compared with those obtained in normal animals maintained on the same diet.

1. With the urine dilution test, when the adrenalectomized animals were well sustained without cortical hormone on the high sodium-low potassium regimen, as dilute or nearly as dilute a urine was obtained as from the intact animals.

2. During hot weather the adrenalectomized animals, deprived of cortical hormone but well maintained on the high sodium-low potassium diet, were unable to produce as concentrated urine as intact animals.

3. In the case of the chloride concentration tests, the maximal chloride concentration was limited in the adrenalectomized animals to levels less than 11.6 grams per liter of urine, while in the normal animal the concentration of chloride in the urine rose to values considerably greater than this level. In these experiments the maximal chloride concentrating ability of the kidney in adrenalectomized animals maintained without cortical hormone on the high sodium-low potassium diet was less than that of the intact animal.

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ABSORPTION AND DISPOSITION OF GLUCOSE IN THE CHICK

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While extensive studies have been reported on the absorption and disposition of carbohydrates from the gastro-intestinal tract of mammals, very few quantitative studies have been made in birds.

Henry et al. (1) determined the absorption coefficient for glucose in adult fowls. These authors did not describe their technique in detail but presumably followed the Cori (2) procedure. Glucose solutions varying in concentration from 13.5 per cent to 54 per cent were used for an absorption period of 20 minutes. The absorption coefficient found for the 3 solutions were, respectively, 209, 204 and 140 mgm. per 100 grams per hour. They found the absorption rate to vary with the concentration of glucose administered, but it should be pointed out that values obtained for such short absorption periods are likely to be unreliable.

Emslie and Henry (3), also employing the Cori procedures, studied the absorption of glucose, galactose and lactose in young chicks. The sugars were administered in 56 per cent aqueous solution and absorption periods of 1.5, 3.0, 4.5 and 6.0 hours were allowed. These workers found the three sugars to be absorbed at the following relative rates: glucose > galactose > lactose. There was a progressive decrease in the rate of absorption of each sugar with time, that of glucose decreasing from 655 mgm. at 1.5 hr. to 245 at 6.0 hrs.

A comparison of the relative velocities of the absorption of different sugars in the rat and pigeon is contained in a brief note by Westerbrink (4). This author has carried out experiments on rats and pigeons which for 5 days had been on a diet consisting only of fat, casein and salts (no vitamins). The rats showed the following series of relative absorption rates: d-galactose: d-glucose: d-fructose: d-mannose: l-xylose: l-arabinose: 108: 100: 42: 15: 13: 2; the pigeons 115: 100: 55: 33: 33: 16. The results with the rat were similar to those of Cori and there was observed no difference in relative absorption rates between the rat and the pigeon.

Recently Morando (5) has reported that ligation of the pancreatic ducts or pancreatectomy in ducks and pigeons was followed by a reduction of intestinal glucose absorption.

In the present investigation the rate of absorption of glucose was studied in young White Leghorn chicks varying in weight from 150 to 250 grams. In addition some information was also obtained on the disposition of the absorbed glucose by observing the changes in the levels of blood glucose, muscle and liver glycogen.

[1] The data forming the basis of this paper were taken from a dissertation submitted by W. R. C. Golden in partial fulfillment of the requirement for the degree of Doctor of Philosophy, Yale University, 1940.

PROCEDURES. The selection and care of the experimental chicks as well as the chemical methods employed are described elsewhere (6).

The general procedure employed for the study of the absorption rate was essentially that outlined by Cori (2) for the study of absorption in the rat.

Merck's reagent grade d-glucose, dissolved in distilled water was used and the chicks were fasted 24 to 30 hours before the administration of glucose. A no. 8 (French scale) rubber catheter attached by means of a shortened 15-gauge hypodermic needle to a syringe of 5 to 10 cc. capacity was used to administer the glucose.

After filling with the glucose solution the catheter was gently inserted into the esophagus and pushed downward until the end was palpable in the crop. The full capacity of the syringe was then delivered into the crop of the chick and the catheter immediately withdrawn. In some instances the end of the catheter went past the crop into the proventriculus. In such cases, the glucose solution must have been delivered into the proventriculus and gizzard but, since in the fasted chick the crop discharges material almost immediately into the gizzard (1), the glucose solution must have arrived at the absorbing portion of the gut in either case at approximately the same time.

To measure the quantity of glucose given, a similar charge from the syringe was delivered into a volumetric flask, diluted to volume and aliquots were taken for analysis.

The time of delivery of the glucose solution into the crop of the chick was recorded and 5 minutes before the expiration of the absorption period the chick was anesthetized, with nembutal given intraperitoneally. When anesthesia was complete, the upper end of the esophagus and lower end of the rectum were ligatured to prevent escape of fluid during manipulation. Then, in successive order, a sample of pectoral muscle and a portion of liver were taken in the usual manner for glycogen determinations and following this, blood was obtained.

As soon as the tissues and blood were obtained, the esophagus was severed anteriorly and the rectum posteriorly to the respective ligatures. The entire gastrointestinal tract was then drawn out, stripped of adhering mesenteric tissues, and transferred to a dish containing about 100 cc. of hot water to which had been added a liberal quantity of powdered sodium fluoride. The entire tract was slit lengthwise and then successively extracted with 4 or 5 portions of hot water, which were transferred to a 500 cc. volumetric flask. Twenty-five cubic centimeters of 10 per cent $ZnSO_4 \cdot 7H_2O$ and 25 cc. of 0.5 N NaOH were next added in respective order with thorough mixing after each addition. The mixture was cooled, made up to volume with water, and after thorough mixing was filtered and a small portion of the filtrate was collected for glucose determinations. Finally, the residual portion of the liver not used for glycogen analysis was taken from the chick and weighed so that the total weight of the liver would be known.

The rate of absorption of the glucose was expressed as the "absorption coefficient" according to Cori's definition: that is, the number of milligrams of glucose absorbed per hour per 100 grams body weight (fasted).

EXPERIMENTAL RESULTS AND DISCUSSION. Before studying the rate of absorption of glucose from the intestinal tract of the fasted chick according to the procedure described above, two questions relating to the method employed had first to be answered. They were:

1. Does the gastro-intestinal tract of a 24-hour fasted chick contain significant quantities of reducing material?

2. Can administered glucose be recovered quantitatively if no time is allowed for absorption to take place?

The first question was answered by removing the intestinal tracts of several fasted chicks, extracting them, precipitating the proteins as described and analyzing the filtrates so obtained. The results indicate that the reducing material of the intestinal tract recoverable by this procedure is negligible. The actual quantities, expressed as glucose, found in 6 chicks were 20, 30, 23, 13, 15 and 10 mgm. No correction for this amount was applied in the calculations.

The answer to the second question was sought in the following manner:

A known amount of glucose was discharged into the crop of each of several heavily anesthetized chicks on the presumption that absorption would be very slow under such conditions. The intestinal tracts of the birds so treated were removed immediately following the administration of the glucose and extracted and analyzed in the usual manner. The glucose recovery values for 3 chicks were 95, 96 and 98 per cent. Emslie and Henry (3) have performed a similar experiment, with the exception that their birds were not anesthetized. They obtained recovery values ranging from 86 to 95 per cent. Some of the lower recoveries may be explained, as these authors suggested, by the fact that in the non-anesthetized birds absorption of the glucose may have already commenced. On the basis of the foregoing evidence it was safe to assume that the difference between the quantity of administered glucose and residual glucose found in the intestinal tract after a fixed time interval was the amount of glucose absorbed in that time.

In the present investigation, absorption of glucose was allowed to proceed for 1-, 2-, 3- and 4-hour periods, in 4 respective groups of birds. In addition to the absorption rate, the changes in the blood sugar and glycogen levels were also determined.

The results of these experiments are summarized in table 1.

An examination of the absorption rates for the four absorption periods would indicate a gradual decrease in the rate with time. This trend, however, is not marked and a statistical analysis of the differences in rate between each group indicates a high probability that they could have occurred by chance variation. This is in contrast to the findings of Emslie and Henry (3) who reported a striking decrease in absorption coefficient from 655 for 1.5 hours to 245 for 6 hours, expressed as milligram per 100 grams per hour.

The overall mean absorption rate for all 4 groups listed in table 1 is slightly over 400 mgm. This is more than twice the rate found in rats of similar size (2) and over 4 times the rate reported for dogs by Trimble, Carey and Maddock (7).

Although these experiments were not extensive enough to study the effect of the concentration of the administered glucose solution on the rate of absorption it is apparent that the slight differences observed cannot be related to the concentration. For example, the concentrations of glucose administered to groups 1 and 2 were respectively 30 and 43 per cent, while the respective absorption rates for these groups were 436 and 430 mgm. Since, in all of the groups, this was the greatest difference in concentration and the least difference in absorption rate, the conclusion is probably justified that moderate differences in concentration are without effect on the rate.

It is difficult to draw any definite conclusions from the response of the blood sugar to the administered glucose. At the end of 1 hour all of the birds in that group showed a uniformly high blood sugar. The 2-hour group showed an inexplicable drop in the blood sugar level. Since the glucose was being absorbed at the same high rate, it might have been expected that the high level

TABLE 1

Absorption of glucose from the intestinal tract

ABSORPTION TIME	NO. OF BIRDS	LIVER GLYCOGEN	MUSCLE GLYCOGEN	BLOOD GLUCOSE	ABSORPTION COEFFICIENT	PER CENT ABS. GLUC. AS LIVER GLYCOGEN	VOL. AND CONC. OF ADMINISTERED GLUCOSE
		mgm. per cent	*mgm. per cent*	*mgm. per cent*			
1. One hour	9	1448 ± 112	915 ± 54	570 ± 22	436 ± 26.6	7.4	5 cc.—30%
2. Two hours	8	3494 ± 296	1150 ± 57	403 ± 38	430 ± 10.7	11.8	5 cc.—43%
3. Three hours	14	4597 ± 175	1344 ± 115	548 ± 42	408 ± 15.3	11.3	10 cc.—35%
4. Four hours	13	5862 ± 223	1306 ± 118	367 ± 35	385 ± 15.4	12.3	10 cc.—40%

of the blood sugar would have been maintained. At the end of 3 hours there was again a rise in the blood sugar, almost to the level of group 1. This was then followed by another drop at the end of the fourth hour. If we attribute the decrease in blood sugar at the end of the second hour to the relatively greater deposition of liver glycogen that took place during that time, the same reason would not apply for the decrease in the blood sugar level at the end of the fourth hour. During this last hour, roughly, the same amount of liver glycogen was deposited as during the third hour, yet in this time the blood sugar fell from a mean level of 524 to 367 mgm. per cent. Probably many of the birds excreted glucose during the absorption period. In the absence of any knowledge of the quantities of sugar excreted and of the amount of sugar burned by these birds, it would be futile to speculate further regarding this fluctuation in the blood sugar curve. In a similar experiment on rats, Cori (8) found that the blood sugar curve reached a maximum at the end of 2 hours and then fell off slightly but consistently each hour for the ensuing 3 hours. Nevertheless, at the end of the fifth hour the blood sugar was still at a higher level than it had attained in 1 hour. In spite of the difference in species, it seems that qualita-

tively, at least, the same type of curve should have been obtained with the chicks.

The rate of deposition of liver glycogen appears to have been greatest during the second hour. Since it has been shown (6) that the fasting control level of liver glycogen is about 350 mgm. per cent, then the quantities of liver glycogen deposited during the first, second, third and fourth hours were in round numbers, 1100, 2000, 1100 and 1200 mgm. per cent, respectively. These quantities, if plotted, would give roughly an "S" type of curve. It is interesting that Cori in the paper cited above also obtained a similar curve for the deposition of liver glycogen, with the chief difference that the third hour was the period of greatest deposition.

Finally, mention should be made of the changes observed in the muscle glycogen level. When it is recalled it has been found that the muscle glycogen of fasted control birds averages 775 mgm. per cent and that of normal fed birds about 1100 mgm. per cent (6), it is apparent from the values in table 1 that only 2 hours were required to replenish the muscle glycogen stores. Within the next hour, the glycogen had increased to supernormal levels and was so maintained during the ensuing hour. It is also of interest to mention that 5 of the individual values for birds in groups 3 and 4 of table 1 ranged from 1810 to 2390 mgm. per cent. As far as we are aware these are the highest muscle glycogen values reported for any avian species and perhaps for mammals also.

An attempt has been made to estimate the amount of absorbed glucose which could be accounted for through glycogen formation. The increase of muscle glycogen observed in the pectoral muscles may have been greater than the increase in the other skeletal muscles. However, if the assumption is made that the observed increase is representative of the entire musculature, then it is possible to arrive at an estimate of the maximum quantity of glucose deposited as muscle glycogen.

A 200 gram chick has about 50 grams of muscle.[2] During 4 hours of glucose absorption the pectoral muscle glycogen increases about 500 mgm. per cent. If this increase is representative for the entire musculature, then a total of 250 mgm. of glycogen would have been deposited. In 4 hours a 200 gram chick will have absorbed 3200 mgm. of glucose (an absorption coeff. of 400). Accordingly a maximum of 7.5 per cent of the absorbed glucose would be accounted for as extra muscle glycogen. Analysis of the liver (table 1) shows that in 4 hours 12.3 per cent of the absorbed glucose is deposited as glycogen and this added to the quantity estimated to be deposited as muscle glycogen shows that not more than 20 per cent of the absorbed sugar can be accounted for in this manner. This is in striking contrast to the 24-hour fasted rat which deposits in the muscle 25 per cent and in the liver 17 per cent of the glucose absorbed in 4 hours (Cori and Cori, 9), a total of 42 per cent, or more than twice the maximum quantity estimated for the chick. While the chick may have a relatively greater amount of glucose distributed in its body water, by far the largest proportion of the absorbed glucose must be accounted for by excretion, oxidation or conversion to

[2] This value was found by direct determinations on another series of birds.

fat. No measure of the excretion could be obtained, but it is extremely unlikely that the glycosuria could have been sufficiently intense to have accounted for very much of the absorbed glucose. Consequently, it would appear that either the chick has a much greater capacity for glucose oxidation than the rat or else is able rapidly to convert a considerable proportion of administered carbohydrate into fat.

SUMMARY

The Cori technique has been employed to study the rate of intestinal absorption of glucose in the chick. A rate of 400 mgm. per hour per 100 grams of body weight has been found. This rate appears to remain constant (within experimental error) over a 4-hour period and does not seem to be affected by moderate differences in the concentration of administered glucose. After 4 hours of absorption the liver glycogen had risen to a level of almost 6 per cent while the muscle glycogen rose to about 1300 mgm. per cent. Of the glucose in 4 hours, 12 per cent could be accounted for as increased liver glycogen but not more than 8 per cent was estimated to have been deposited in the muscles. It is suggested that the chick possesses either a relatively intense ability to oxidize glucose or is able to convert a considerable proportion of absorbed glucose into fat.

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OBSERVATIONS ON THE ACCURACY OF THE THERMOSTROMUHR[1,2]

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Over two and one-half years ago a method was sought to quantitate blood flows in the coronary arteries of normal unanesthetized dogs. While the orifice plate meter (1), and the rotameter (2) were suitable for recording phasic and mean flow in acute experiments, the thermostromuhr seemed the best available device for use in chronic experiments. In attempts to use it, certain difficulties have been encountered which seem insurmountable, and which indicate that under most experimental conditions it cannot be used for quantitative measurements. Since the thermostromuhr is so widely used in just this way, it seems worth while to report our experiences with the method.

PROCEDURE AND METHOD. Direct current thermostromuhrs (3) were either bought or constructed according to the specifications of Baldes and Herrick. This type of unit was adopted because of its simple structure. Following standard practice, the units were calibrated *in vitro* before application to arteries in animals. In all uses of the thermostromuhr corrections were made for the null point on the galvanometer before and after each determination. In any one unit, and for a given experiment the heating current was kept constant; in different units this varied from 200 milliamperes to 350 milliamperes. For the calibration, the unit and long, snug-fitting artery segment were connected to an artificial circulation system which produced a flow pattern, as revealed by the orifice plate meter, similar to an aortic pressure curve (cf. fig. 3, part C *D-2*). The peripheral resistance was never less than 60 mm. Hg, and the calibration was made at room temperature with whole blood of known viscosity (4) rendered non-coagulable with heparin and pontamine fast pink. The actual flow was determined either by a graduate or, more usually, by a calibrated rotameter in series with the unit. The thermostromuhr unit was either placed in a moist chamber[3] or surrounded by saline or cotton saturated with saline.

Following the *in vitro* calibration, the units were applied with aseptic precautions to the arteries of dogs (in most instances the coronary arteries). When the coronaries were used, special precautions, such as the stitching of the unit and its overlying pericardium to the epicardium, minimized movement and angulation of the unit with respect to its artery. Drainage was largely eliminated by periodically packing the lead wire incisions with sulfanilamide.

[1] The expenses of this investigation were defrayed, to a large extent, by a grant from the Commonwealth Fund.

[2] A preliminary report of this work was presented before the American Physiological Society at Chicago, April 15, 1941.

[3] The unit and its contained artery segment were supported in a closed chamber on the floor of which was placed cotton saturated with saline.

Galvanometric readings were taken in 36 dogs (generally on alternate days). Such readings were assumed to represent the same flows as indicated in the *in vitro* calibration. The experiment was generally terminated by calibrating the unit in the above circulation system with the animal's own blood, after careful removal to it of the unit with its artery and surrounding tissue. On occasion, the final calibration was obtained *in situ* by anesthetizing the dog and then connecting a calibrated rotameter peripheral to the unit and varying the flow by means of a screw clamp on the rotameter.

RESULTS. In some of the first experiments a number of days after application of the units, the Bakelite blocks developed electrical leaks and the lead wires frequently broke at the point of emergence from the chest wall. Accordingly, the original braided tinned copper leads were replaced with 8–10 strands of no. 36 or no. 37 annealed silver wire, and the covering varnish or lacquer was replaced by Bakelite lacquer XV-14463. By this procedure electrical and mechanical trouble was largely eliminated in chronic experiments (1–2 mos.).

In some dogs the empirical flow readings derived from the previous *in vitro* calibration were reasonably constant from day to day. In figure 1-A are presented the flow data obtained with a unit that had been on the left circumflex artery of a dog for 30 days (curve *F-2*). After the fifth day the empirical flow varied from 39 to 63 cc./min. Such flows might be the reasonable expectancy in the left circumflex. However, in other dogs such flow figures, although reasonably constant from day to day, vary from 20 to 150 cc./min. in different dogs (12–18 kgm. in weight) (*F-3*, 1). It seems highly improbable that flows in a given coronary branch of different well-trained dogs could vary by so large an amount. Also, since *in vitro* calibrations taken at the beginning and end of a chronic experiment were found to vary considerably (up to 50 per cent) any blood flow values thus derived are questionable. Hence, tests were made to determine the trouble and if possible to eliminate it.

In such tests the unit has been studied in the living dog, and also in an artificial circulation system, to obviate the change of more than one variable at a time.

The first test was the comparison of the *in vivo* and the *in vitro* calibrations of the same thermostromuhr unit. These curves are presented in figure 1-B. For all curves the actual flow was determined by a rotameter inserted peripheral to the unit. For curve 1 the unit was applied to the carotid of an anesthetized dog and the incision closed. The peripheral end of the rotameter was connected to the central end of the jugular vein on the opposite side, and the flow was varied by a screw clamp peripheral to the rotameter. For curve 2 (taken about 15 min. later) an *in vivo* moist chamber was created without disturbing the artery, by careful removal of all tissue and fluid from contact with the artery and by closing the incision with skin and moist towels. For curve 3 (about 30 min. later) the same artery (12 cm. long) with its attached unit was removed to the moist chamber of a circulation system, connected to two 18 inch plastic cannulae without apparent constrictions or deformations, and then calibrated with the same dog's blood at room temperature. These curves differ by about 50 per cent. Obviously, the use of a calibration obtained in a moist chamber is restricted and may not be used to derive flow values in animals.

The next step was the investigation of the cause for the difference between the calibration curves obtained in the animal (curves 1, 2; fig. 1-B). Accordingly, the effects of tissue components *per se* together with the movements of extra-vascular and intra-vascular fluid upon the calibration of the thermostromuhr were tested.

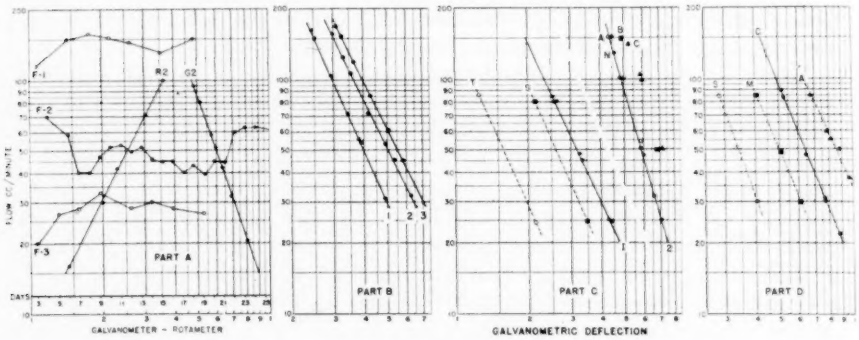

Fig. 1. Part A. Graphs showing blood flow values in left coronary arteries of different dogs calculated from *in vitro* calibration curves of thermostromuhr. *F-1*, *F-2*, *F-3*, empirical flow values in dogs (D-48, 46, 49) weighing 18.0, 12.0 and 12.0 kgm. respectively. *F-1* is based on G-2 (*in vitro* thermostromuhr calibration) and R-2 (calibration of rotameter used to calibrate thermostromuhr). Ordinate—coronary flow cc./min. Abscissa—galvanometric deflection in 100 mm. Rotameter—in millimeters. Days— as indicated.

Part B. Curves showing difference between calibrations of thermostromuhr in a moist chamber and *in vivo*. Curve 1, unit on carotid artery of anesthetized dog and surrounding skin and muscle in contact with unit. Curve 2, same set up 15 minutes later, except that *in vivo* "moist chamber" created. Curve 3, unit and artery of curve 2 carefully removed to pump system and calibrated at room temperature with same dog's blood. For all curves flow through unit measured by rotameter connected peripheral to it.

Part C. *In vitro* calibration curves obtained on same thermostromuhr unit with different environmental conditions prevailing. Curve 1, obtained on unit after it had been applied to femoral artery of dog for two weeks and then removed to calibration system with its contained artery and surrounding tissue. Points S—same but after artery stretched about 10 per cent. Points T—same but tissue carefully clipped from unit and artery. Curve 2—unit calibrated in a pump system surrounded by saline, *N* (dots); ascitic fluid *A* (open circles); whole blood, *B* (squares); blood diluted 1:3, *C* (triangles).

Part D. Unit calibrated in pump system and surrounded successively by saline soaked cotton, *C*; fresh muscle tissue, *M*; saline, *S*; moist chamber, *A*.

Effects of environmental changes. In an artificial circulation system maintained at room temperature, the calibration curves are essentially identical when saline or ascitic fluid surround the unit, but if the surrounding fluid is more viscous (whole blood or blood diluted 1:3), shifts of 50 to 100 per cent in the calibration curve occur (fig. 1-C, curve 2, points *B*, *C*). If a unit with its artery segment is surrounded by cotton soaked in saline, and then in succession by fresh moist tissue, saline alone, and finally, a moist chamber, curve *C* shifts to curves *M*, *S* and *A* respectively (fig. 1-D).

To test further the "tissue effect" in animals, units have been applied to the femoral arteries of a number of normal dogs for 2 weeks, and then the unit and artery, together with the surrounding tissue, have been removed to the calibration system. Curve 1, figure 1-C, was obtained from such a unit. After tissue removal from the region of the unit by careful dissection, calibration gives points *T*. Similar shifts in the calibration curve have been obtained following tissue removal from the unit applied to a femoral artery for two weeks and then calibrated *in situ*.

Movements of intra-vascular fluid. A thermostromuhr is directly affected by the flow of blood in the capillary bed and large vessels around it, as indicated by changes in galvanometer deflection without concurrent changes in blood flow through the unit. Representative data in figure 2-A have been taken from different experiments and arranged so that the same apparent shifts in flow occur as in each experiment. To obtain the data, the unit and its contained excised artery were buried between muscle layers of an anesthetized dog and then connected with the calibration system and rotameter, the whole ensemble being maintained at essentially constant temperature in a water bath. The flow through the calibration system and unit was maintained constant, as indicated by the rotameter, and flow alterations in the surrounding tissue were induced progressively by mechanical constriction of its nutrient artery (iliac) or by vasodilator or constrictor drugs. The apparent flow as read from the galvanometer deflection shifts about 30 per cent (dots and circle) while the actual flow through the unit is unchanged.

Similarly, if in the same arrangement the flow through the unit is again kept constant and the cold junction of the unit is purposely placed close to the femoral artery of the host dog, reduction of flow through this same artery causes shifts in the galvanometric deflection (triangles) indicating apparent flow increases of 40 to 300 per cent through the unit, despite the constant reading of the rotameter. Similarly, if the area over the hot junction is placed in contact with the femoral artery of the anesthetized dog and blood flow through that artery is reduced, errors of comparable magnitude, (squares) but in the reverse direction, are noted. The same results can be obtained in a calibration system if the rate of fluid flow is changed through a small rubber tube placed in the same fluid bath as the unit, and close to the latter.

Movements of extravascular fluid. Such movements can also significantly affect the calibration curve. Ten to fourteen days after a unit had been applied to the iliac artery of a dog, the dog was anesthetized and the unit area exposed and observed. Visible fluid movements about the unit induced by intestinal activity resulted in marked alterations of the galvanometric deflection. Similar results were obtained in a calibration system by stirring or tilting the chamber fluid.

While the variations in external environment adequately explain the differences between curves 1 and 2 in figure 1-B, to account for the difference between the moist chamber calibration in an artificial circulation system (curve 3) and the *in vivo* calibration (curve 1), additional factors such as differences in

heart rate, stroke volume, viscosity, pattern of the flow curve, in the vessels used and in their contact with the thermostromuhr unit must be considered. Some of these variables are quite effective, others are not.

Temperature, heart rate, and stroke volume. These are potentially effective variables which have little or no effect on the accuracy of the thermostromuhr unit. In figure 2-B the calibration curves taken in an artificial circulation system indicate that variations in blood temperature of ± 4°C (curve 1), alterations of

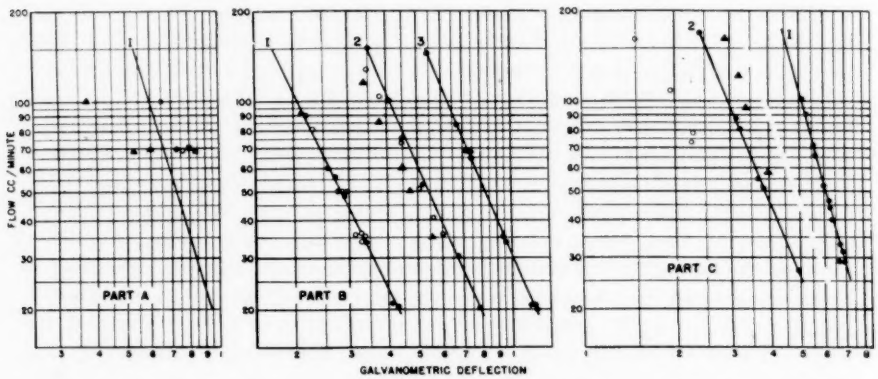

Fig. 2. Part A. Effect of change of external environment in living animal on calibration curve of thermostromuhr. Curve 1, assumed calibration curve from which recorded points are placed so that they show same percentage error as in original experiments. Unit and contained artery segment embedded between muscle layers in leg of anesthetized dog and connected to pump system. Dots, iliac artery partially constricted. Circle, nitroglycerine injected by vein. Triangles, cold junction of unit placed near femoral artery with iliac artery flow reduced mechanically. Squares, same but with hot junction near femoral artery.

Part B. Effects of alteration of temperature, (curve 1), viscosity (curve 2), and heart rate and stroke volume (curve 3) on calibration curves obtained in a moist chamber. Dots —control points. Temperature change from 29°C (dots) to 24°C (circles). Specific viscosity change from 8.4 (dots) to 5.3 (circles) to 3.1 (triangles).

Part C. Curve 1, calibration points obtained with blood at room temperature over a four-hour period. Thermostromuhr surrounded by saline in an artificial circulation system. Curve 2, calibration of unit in moist chamber showing effects of angulation and movement of unit on its contained artery. Dots, control points. Circles, angulation. Triangles, unit movements.

heart rate (pump rate) from 90 to 300 per minute, and gross changes in the pump stroke (curve 3) do not materially affect the calibration curve.

Blood viscosity. The specific viscosity of blood in normal, unanesthetized dogs, as determined in a previous investigation (4), varies from 3.7 to 7.0, with most of the values falling between 4.0 and 6.0. When the specific viscosity in the circulation system is altered from 8.4 to 2.8 by adding or subtracting red cells or plasma or by adding saline, the calibration shifts about 25 per cent (cf. fig. 2-B, curve 2).

Differences in vessel and contact. Various *in vitro* calibrations of the thermostromuhr unit on the same artery in a calibration system can agree very closely, provided internal and external conditions are unchanged. For example, in figure 2-C, curve 1, with the unit and artery suspended in a moist chamber, the maximal error from the initial calibration curve over a four hour period is 10 per cent.

However, calibration of the same unit applied to different arteries in an artificial circulation system may give calibration curves of considerable difference, all other conditions remaining the same. In figure 3-A, the extremes of three

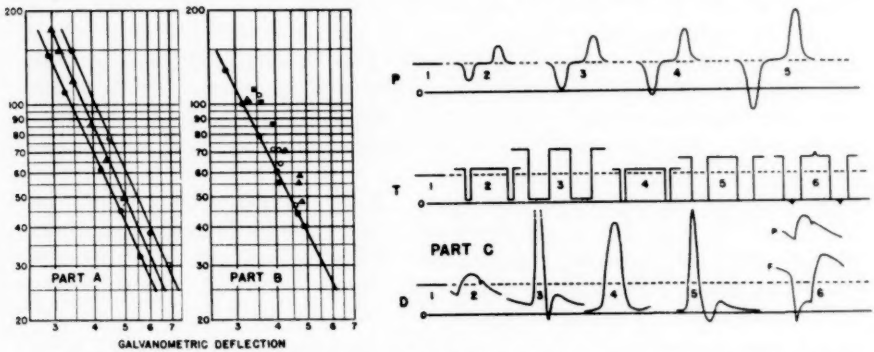

Fig. 3. Part A. Calibration curves obtained with blood at room temperature on same thermostromuhr unit applied to three different arteries in a moist chamber of a circulation system.

Part B. Calibration curve on carotid artery of anesthetized dog with unit surrounded by its normal complement of tissue and fluid. Unit calibrated by rotameter placed peripherally and connected to central end of opposite jugular vein. Dots—initial control points. Squares—aminophylline injection. Triangles—nitroglycerine injection. Circles—control points after drugs.

Part C. Diagrams illustrating various flow patterns produced by a pump system and recorded by orifice plate meter. *D-2, 3, 4, 5* and *6*, flow patterns similar to those existing in carotid, femoral, coronary sinus, femoral, and left coronary respectively, in anesthetized dogs. *P*, aortic pressure. *F*, flow. *O*, zero flow.

typical calibration curves obtained in a moist chamber on the same unit vary by about 40 per cent.

Similarly, movements of the thermostromuhr unit relative to the artery can significantly affect the calibration curve. For the data in figure 2-C, curve 2, the unit was placed on a 12 cm. length of carotid artery in a moist chamber and calibrated. Readings were then made, with the unit placed in different positions along the distal half of the artery, with the artery held at different lengths, or with the unit angulated 20 to 30° with respect to the long axis of the artery. The errors thus induced vary from 22 to 100 per cent. It should be emphasized that movements of the unit on the same artery and the use of different arteries may not always influence the calibration curve.

Similar errors occurred in experiments in which the unit was calibrated against

a rotameter in an anesthetized dog. Attempts to make the contact more permanent by long continued application of the unit to an artery of a dog have proved unsuccessful. Units have been placed on the femoral arteries of dogs for two weeks and then calibrated *in vivo* with a rotameter. Slight changes (10 per cent or so) in the length of the artery (manually induced) give points *S* (30 per cent shift) as compared to control curve 1, in figure 1-C.

Pattern of the flow curve. As demonstrated by the orifice plate meter, the contour of the flow curve in various arteries of the anesthetized dog is different. In the right coronary (5) the flow is presumably always forward, although phasic velocity varies greatly. In the left coronary (1, 7) and the femoral artery (6) of the anesthetized dog, and in the human femoral (8) the presence of zero flow and back flow components is the usual finding, while in the carotid and numerous other arteries these flow components can appear.[5] Such phasic alterations in velocity and direction of flow could conceivably affect the accuracy of the unit. This has been tested in the circulation system and in the anesthetized dog.

In a circulation system, the flow curves, as determined by an optically recording orifice plate meter, interposed distal and close to the unit, range from those of linear conformation, high and low velocity components, those with zero and back flows to those simulating a left coronary, carotid, femoral artery and coronary sinus pattern (for sample curves cf fig. 3, part C, *D*).[4] In general, phasic variations of flow velocity during a mechanical cycle do not grossly affect the calibration curve, provided the rate of flow never approaches zero (cf. fig. 3, part C, *P-1, 2, 3; T-1, 2; D-1, 2*). However, if the velocity is relatively low for a considerable portion of the cycle (*T-3*) and particularly if the fast and slow components of the cycle show great differences, as in *D-4*, the galvanometer indicates a somewhat greater flow (fig. 4-A) (circles) than is actually passing through the unit. Introduction of periods of zero flow existing for 10 per cent or more of the cycle (fig 3, part C, *T*-4, 5) reduces the galvanometric deflection considerably, and the error is roughly of the order of 1 per cent for each percent of zero flow in cycle time (fig. 4-A, dots).

When small back flows (2–10 per cent of cycle volume) of short duration (2–10 per cent of cycle time), as in figure 3, part C, *P-4*, are induced or are superimposed on an already existing period of zero flow (fig. 3, part C, *T-6*) the error can be 50 per cent (fig. 4-B). Larger back flows spaced in the same or a slightly longer time period (fig. 3, part C, *P-5*) give larger errors. However, the most striking effects have been seen in flow patterns with small back flow volumes spread over a considerable portion of the cycle. In figure 4-B, the circles, connected by interrupted lines, were obtained with flow patterns in which the portion of the cycle occupied by back flow was about 1 to 30 per cent in cycle time, and less than 10 per cent in cycle volume. The error reaches a few hundred percent. In "synthetic" coronary flow curves (fig. 3, part C, *D-6*) taken from different experiments and with back flows of 3 to 10 per cent of cycle

[4] The flow curves represented were created by a combination of various expedients, the description of which is beyond the scope of this paper.

volume and occupying 3 to 10 per cent of cycle time, the apparent increase in flow varies from 45 to 95 per cent (fig. 4-B).

Similarly, in the anesthetized dog, flow measurements by means of the thermostromuhr on arteries in which back flow is known to exist are attended by large errors. In figure 4, part C, are shown typical results with use on the femoral artery before (point *C*) and during (circles) late stages of the effect of the injection of a small amount of epinephrine (jugular vein) as contrasted with the control curve without back flow (dots and uninterrupted line) obtained by

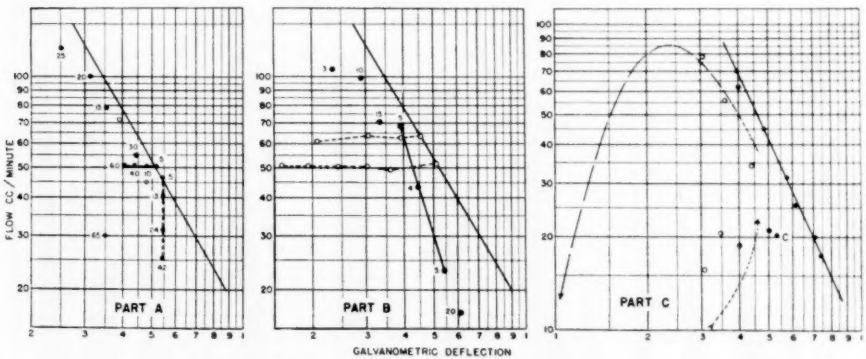

Fig. 4. Part A. Effect of near zero and zero flow on calibration curve of thermostromuhr. Points are from different experiments and curve is assumed calibration from which recorded points are placed so that same percentage error exists as in original experiments. Numbers indicate percent zero flow. Connected points are from same experiment.

Part B. Effect of back flow on thermostromuhr calibration. Long curve—same as in part A. Numbers indicate percent back flow volume. For all dots, back flow occupied from 2 to 10 per cent cycle time. Connected points are from same experiment. Circles, approximately 2 to 10 per cent back flow volume spread over 1 to 30 per cent of cycle time.

Part C. Graph showing observations made with thermostromuhr applied to femoral artery of anesthetized dog. Dots, control points when flow (adjusted by screw clamp) is from femoral artery to femoral vein of same leg and flow pattern contains no zero or back flow. C, normal flow through femoral artery containing periods of zero flow and back flow. Circles, points after zero and back flow increased by injection of epinephrine (0.07 mgm.) by jugular vein. Dotted line, trend of galvanometric deflection and blood flow after epinephrine injection.

shunting femoral artery flow into its femoral vein. (Pattern and actual flow determined by orifice plate meter and rotameter.) A more complete picture (based on a number of experiments) of the trend of the temporal relations of actual flow and galvanometric deflection (interrupted line) throughout the effects of epinephrine injection is further instructive. While the actual flow increases and then decreases, the galvanometric deflection decreases only, and its direction actually reverses with relation to flow. This is manifest by reversal of the slope of the curve. Orifice plate meter records indicate that back flow present before injection diminishes near the beginning of the flow increase, is augmented as flow begins to drop, and becomes maximal at lower flow levels.

This means that the effect of the augmentation of back flow has exceeded the effects of decreasing flow, so that the galvanometer registers a flow increase, when actually a flow decrease exists.

Finally, in the use of the thermostromuhr another potential error exists, which is not revealed by a calibration curve. This is the considerable mechanical limitation of flow by the thermostromuhr *per se* (9).

Discussion. Various types of thermostromuhrs are used to quantitate blood flows. The differential temperature of the two thermocouples, as in-

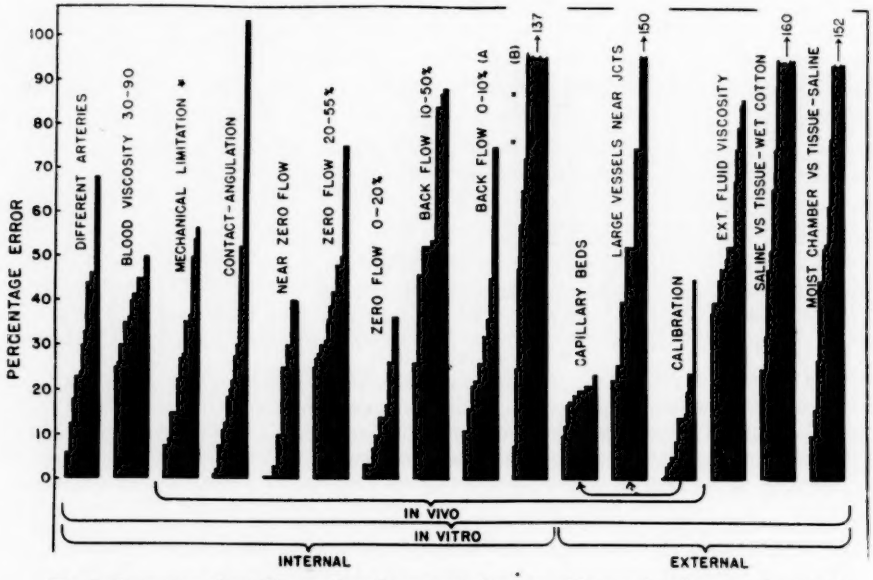

Fig. 5. Summary chart illustrating the extent of errors of internal and external environmental origin which were found to exist when thermostromuhr was calibrated *in vivo* and *in vitro*. Each bar segment represents two or more observations. (A) Back flow 1 to 10 per cent by volume occupying 1 to 10 per cent of cycle time. (B) Back flow 1 to 10 per cent by volume occupying 10 to 30 per cent of cycle time. * Data on effect of mechanical limitation of flow taken from paper of Shipley and Gregg (9).

dicated by the galvanometer, has been assumed to be an accurate index of flow, once the relation has been established by calibration. This assumes that essentially all, or a constant percentage of the heat from the heater enters the blood stream, and that at any one flow no internal or external influence affects this relation. On this basis an *in vitro* calibration of the thermostromuhr should give the correct blood flow values for galvanometric deflections obtained from the same unit applied to a vessel of a living animal.

The data presented here do not substantiate this assumption, but rather indicate that the differential temperature of the couples reflects changes in the internal and external environment of the unit other than those induced by

changes in rate of blood flow alone. For convenience, the percentage deviation of the empirical flow from the actual flow that can be induced by these variables is summarized in figure 5. Among such effective influences are 1, the position of the unit on the artery and its degree of angulation with respect to its contained artery; 2, use of different arteries; 3, blood viscosity; 4, the existence of near zero, zero and back flows in the pattern of the flow curve; 5, character of fluid and material around the unit block, and 6, the flow of extravascular fluid and of blood in the capillary beds and larger vessels near the thermostromuhr. In addition, the thermostromuhr mechanically limits flow through itself, presumably without causing any changes in the calibration curve.

The effects of these different factors may be sizable. Separately, the errors range from 0 to 300 per cent; taken together, the error is fantastic. However, it should be emphasized that in many calibrations, some of the errors will not exist, and the direction and magnitude of the individual error will vary with the individual unit and dynamic conditions prevailing.

In vitro calibrations on the same unit are found to vary with the external environment, and all generally differ from an *in vivo* calibration. Therefore, the flow of blood in an artery of an animal can only by chance happening be determined from a unit applied to it which had been previously calibrated in an artificial circulation system. This is so, because, for the same unit, it is indeed an accident when *in vitro* and *in vivo* environments influence the differential temperature-flow relations in the same direction and to the same extent. It is difficult to understand the contrary viewpoint of some (10, 11) that the practice of obtaining a calibration curve on any vessel in a moist chamber and pump system and applying it to readings made with the unit placed on an artery in a normal, unanesthetized dog permits errors of not more than 10 per cent. The experimental proof for such a view has not yet appeared. Such a view ignores the observations of Baldes (3) that maximal and minimal values in calibration curves for the same unit on different arteries vary by as much as 50 per cent, the observations of Grodins, Osborne, Ivy and Goldman (12) that *in vitro* calibrations on the same unit disagree by sizable amounts from *in vivo* calibrations on the hepatic artery, and, finally, the potent and unpredictable effects on a calibration curve demonstrated here of changes in the external and internal environment.

Our data indicate that the differential temperature of the thermocouples of the thermostromuhr fails to retain its calibrated relation to flow in the presence of flow patterns containing periods of near zero, zero, or back flow, regardless of the application of a unit to *in vitro* or *in vivo* experiments. In the case of back flow the error is many times greater than a simple back flow/total flow ratio would indicate. Since, in acute and chronic experiments, the volume and timing of these flow components change with dynamic conditions, and such patterns cannot be predicted and duplicated in a calibration system, calibration curves with quantitative significance cannot be obtained for units when applied to such arteries either *in vitro* or *in vivo*.

In animal preparations it is also possible that the galvanometric deflection

will not always decrease as the flow increases, and vice versa. In the femoral artery of the anesthetized dog in which back flow has been shown to occur under experimental conditions, injection of a constrictor drug may so augment the back flow that the direction of the galvanometric deflection will be completely reversed with respect to flow. Similarly, instances have occurred in which, after a unit had been applied to a coronary artery for many days, empirical flow values of reasonable expectancy have been recorded which increase upon injection of epinephrine. Yet, autopsy an hour later revealed that the artery had been completely occluded, apparently for some days. In short experiments (a few hours), however, and under most circumstances, the thermostromuhr will presumably indicate correctly directional changes in flow, provided the blood flow is at all times forward, and at no time approaches zero.

In vivo calibration of the unit should remove or minimize some of the errors associated with its use on arteries exhibiting all forward flow, in either acute or chronic experiments. To test this, the units were applied to carotid arteries of anesthetized dogs and the blood flow measured by a calibrated rotameter connected to the artery peripheral to the unit. Since back flow can occur in carotid flow patterns an all forward flow was made certain in these experiments by shunting blood flow (controlled by a screw clamp) from the peripheral end of the carotid to the central end of the severed jugular vein on the opposite side. After the incision was closed and a calibration curve obtained on the unit, the carotid flow and galvanometric deflections were recorded for a period of from 1 to 3 hours. At times during this period the flow was altered by various means, such as drug injection. Any deviation of the galvanometric deflection from the known flow through the unit on the basis of the preliminary calibration curve gives the extent of error due to the change in environment. A typical curve presented in figure 3-B indicates the expected error may be sizable, and varies from 0 to 40 per cent. In long continued experiments (days to weeks) the error of such an *in vivo* calibration would be expected to be much greater because of the unknown amounts of angulation, mechanical reduction of flow by the unit (in many instances enhanced due to thickening of the artery wall), alterations in the external environment of the unit occurring naturally, or that also presumably induced by the anesthetic and anticoagulant used for final calibration, and, finally, possible changes in flow in the vasa vasorum.[5]

The fact that the *in vivo* and *in vitro* calibration curves for any one unit are approximately parallel, although not superimposable, suggests that percentage changes in flow in an experiment can be determined from any one of these curves with fair accuracy. The prerequisite is that in an actual experiment the en-

[5] This is suggested by the observation that the apparent blood flow through a thermostromuhr on a carotid artery in an anesthetized dog may shift appreciably with drug injections, despite the facts that the unit is without contact with the surrounding tissue and fluid ("moist chamber"), any preexisting back flow or zero flow is eliminated from the flow pattern by the procedure described above as used for obtaining the data for fig. 3-B, the artery central to the unit is uncut and the actual flow through the unit is measured by a rotameter and kept constant by a screw clamp on the latter.

vironmental conditions and pattern of the flow curve are as well stabilized as when under the rigidly controlled conditions for calibration. Presumably, this state is approached in experiments in which flow in a vessel is at all times forward, and circulatory conditions in the animal are undisturbed. However, it is difficult to conceive of changes in blood flow through a vessel, resulting from drug injection, alterations of blood pressure, heart rate, etc., without simultaneous changes in the immediate environment and/or flow pattern, with their attendant large effects on the calibration curve (cf. fig. 4, part C). Since these changes necessitate the establishment of a new equilibrium, the true calibration curve no longer remains fixed, but may be actively shifting either to left or right, coincident with, but not caused by the flow change in the vessel. Thus, the true moment to moment relation of flow to galvanometric deflection which is applicable to a given series of flow changes may no longer be straight or parallel to any previously or subsequently obtained calibration curve. Hence, the percentage change in flow cannot be determined.

SUMMARY AND CONCLUSIONS

Direct current thermostromuhrs, with improved lacquers and lead wires, have been applied to the arteries of dogs in acute and chronic experiments. The units have been calibrated in an artificial circulation system, or *in situ* by means of a rotameter. Tests made in the circulation system and in the anesthetized dog indicate that blood flow values in chronic experiments, as read from an *in vivo* or *in vitro* calibration, may be highly inaccurate because the relation of the galvanometric deflection to flow will vary with 1, the artery used, and its degree of stretch; 2, the position and degree of angulation of the unit with respect to its contained artery; 3, the presence or absence of near zero, zero or back flow in the flow pattern of the metered fluid; 4, composition of the immediate environment; 5, movements of extra- and intra-vascular fluid in the environment, and 6, viscosity of the metered fluid.

In acute experiments application of *in vitro* calibrations is not justified for the same reasons. *In vivo* calibrations may allow a semi-quantitative measure of flow provided environmental influences are eliminated and flow is at all times foreward.

Since, in any one experiment, the occurrence and magnitude of the effect of most variables can neither be controlled nor predicted, the conclusion is reached that any interpretation of galvanometric deflections obtained with a thermostromuhr in acute or chronic experiments in terms of absolute blood flow or percentage change in flow is open to grave question.

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OPERATIVE MECHANISM OF SOME ERRORS IN THE APPLICATION OF THE THERMOSTROMUHR METHOD TO THE MEASUREMENT OF BLOOD FLOW[1,2]

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In a preceding paper (1) a practical evaluation of the accuracy of the thermostromuhr (Baldes and Herrick type) has revealed that, by changes of environment and flow pattern, sizable errors are introduced into its quantitation of blood flow. To clarify the *modus operandi* of these factors the mechanism whereby changes in galvanometric deflection reflect changes in flow was studied.

Since, with a thermostromuhr of this type, the galvanometric deflection varies with the differential temperature of the two thermocouples, but the amounts of deflection and flow vary in opposite directions, it is logically concluded that the higher the flow the less temperature difference exists between the two thermocouples. To evaluate the contribution of individual couple temperatures to this differential temperature a special thermostromuhr unit was constructed for this and other studies.

In substitution for the ultimate use of a blood vessel a thin-walled glass tube 12 cm. in length (O.D.—2.23 mm.; I.D.—2.10 mm.) was permanently built into a conventionally shaped unit (10 mm. broad, 10 mm. long, and 3.5 mm. thick). The heater and couple elements were held in contact with the outside of the tube during the hardening of the lacquer. The Bakelite block (BT48-005) and lacquer (BL-3128) used gave essentially a transparent unit. In addition, the wiring was modified to the following extent: The constantan connection between couples within the block was eliminated by running the constantan wires directly through the block to the outside, where connections could be made for recording the temperature of each junction separately. Elimination of this connection removed a potential pathway for direct heat transfer to the couples from the heater area. Also, the couples were placed equidistant from the flat sides of the unit on the floor of the lumen where they would presumably be least vulnerable to environmental influences. A reference thermocouple placed in a thermos bottle completed the galvanometer circuit.

The unit, surrounded by dry cotton, was connected in a pump system and perfused with acacia solution (spec. visc. 4.0—4.5) at room temperature. The sensitivity of each couple was determined with reference to the thermocouple in the thermos bottle.

[1] The expenses of this investigation were defrayed to a large extent by a grant from the Commonwealth Fund.

[2] Preliminary reports of this work were presented before the American Physiological Society at the Boston Meeting, April 1942 and before the Cleveland Section of the Society for Experimental Biology and Medicine December 12, 1941.

In figure 1-B are shown the elevations in temperature [above that of the perfusing fluid and environment (room temperature)] at the two thermocouples with three heating intensities and at several rates of flow through the unit. Also shown are what could be called calibration curves representing the differential temperatures computed by subtraction. In figure 1-A are plotted corresponding temperatures of the perfusion fluid opposite the two couples as determined by exploring thermocouples inserted in the glass tube to the level of the standard couples and in contact with the wall immediately over each.

For purposes of revealing the significance of these curves, let us assume that a hypothetical state exists within a unit in which the cold or upstream junction

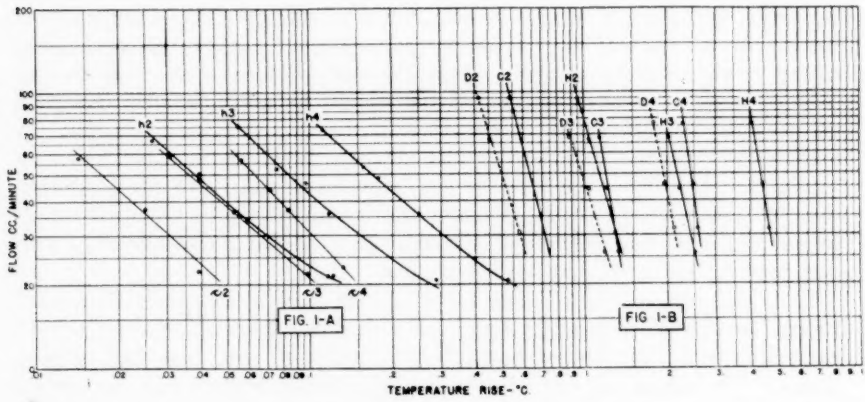


Fig. 1. *A*. Curves indicating temperatures of fluid (above that of incoming fluid) measured immediately opposite the hot, *h*, and cold, *c*, thermocouples when thermostromuhr unit was perfused with acacia solution (spec. visc. 4.0–4.5) at room temperature with heater currents of 200 (2), 300 (3), and 400 (4) milliamperes. Ordinate: Flow in cc./min. Abscissa: temperature rise °C.

B. Curves of individual thermocouple temperatures of same thermostromuhr with differential temperatures at different flows and heating intensities. *H*, hot thermocouple; *C* cold thermocouple; *D*, differential temperature. 2, 3, and 4—same as in figure 1-A. Ordinate and abscissa—same as in figure 1-A.

and incoming fluid are of the same temperature, and the hot or downstream junction faithfully records all, or a constant proportion of the temperature rise sustained by the fluid as it passes the heater. The differential temperatures obtained would show that as the flow doubled the deflection would decrease by one half, thereby affording maximal "sensitivity." That such optimal conditions do not exist in ordinary practice is at once apparent (fig. 1-A, 1-B). First, the temperature of the cold junction is not the same as that of the incoming fluid. Second, the temperature of the hot junction does not vary inversely as the flow, and therefore is not entirely influenced by the rate of flow alone. And third, the temperature of each junction is considerably in *excess* of the temperature of the fluid passing by each (C_2 versus c_2, H_3 versus h_3, etc.). The concept

of a conceivable working distribution of heat within a thin sagittal section is diagrammed in figure 2.

It would seem, therefore, that the great bulk of heat reaching both of the unit's thermocouples is supplied directly via the block. And since both couples are hotter than the passing fluid it follows that changes in the rate of flow alter the differential temperature through corresponding changes in the rate of *cooling* of both couples. The extent of cooling depends primarily upon two interdependent factors: first, how hot the couple is (determined by the rate heat is supplied from the block and the rate at which it is removed by the fluid) and, second, how cool the fluid is (determined by the temperature of the fluid enter-

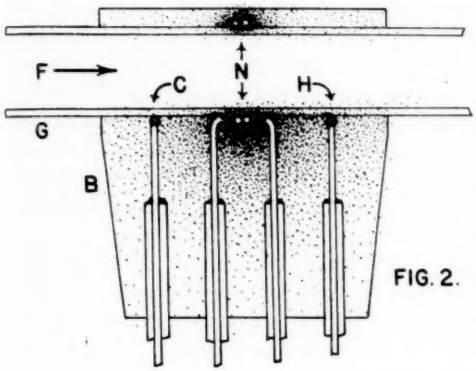


FIG. 2.

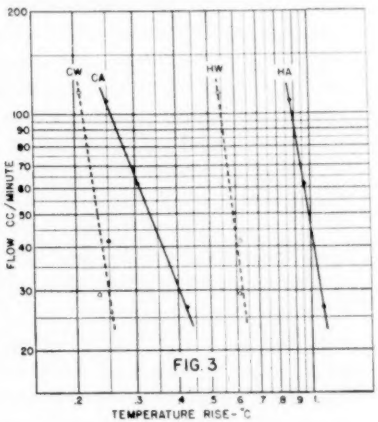


FIG. 3

Fig. 2. Diagram of concept of gross heat distribution within a thin sagittal section of thermostromuhr described in text. Density of stippling indicates relative temperature elevation. *F*, fluid; *G*, glass tube; *B*, Bakelite block; *C*, cold junction; *H*, hot junction; *N*, heater.

Fig. 3. Curves showing temperature rise of thermocouples applied to exterior of thermostromuhr unit over the respective sites of the internal thermocouples with external environments of air and of water. *H*, thermocouple over hot junction; *C*, thermocouple over cold junction; *A*, temperature curves with unit surrounded by air (dry cotton). *W*, unit surrounded by water (water saturated cotton). Ordinate and abscissa—same as in figure 1.

ing the unit and the temperature rise sustained as it approaches the couple area).

In general, the heat gradient through the block extends centrifugally, with the heater its highest point (fig. 2). During flow the axial ends of the unit with their contained thermocouples, even though carefully placed equidistant from the heater, will not have the same temperatures because of the greater cooling effect of the unheated incoming fluid upon the upstream end. The extent to which the temperature of the fluid opposite each junction influences the respective junction temperatures with a given change in flow is indicated by the different slopes of the junction temperature curves at any given heating intensity (C_2 versus H_2, etc.) (fig. 1-B).

The foregoing considerations indicate that a calibration curve represents the relation of the rate of flow to the differential temperature of two thermocouples whose individual temperatures are determined by the respective *rates at which they are cooled* which, in turn, are derived from the heat gradients between the heater and fluid stream at the sites of the thermocouples.

Environmental influences, externally induced. The above study demonstrates that the influence of the heater extends considerably beyond its immediate environment. A sizable temperature elevation was observed at the upstream junction, in spite of its proximity to the incoming fluid (fig. 1-A). This led to the belief that a variable amount of heat would be lost to the outside environment through the block, and thereby alter the thermal equilibria at the two junctions. Accordingly, two thermocouples were lacquered to the outside of the block immediately over the sites of the internal couples. After calibration of the couples local temperatures were then recorded under rigidly controlled conditions with the unit surrounded first by a layer of dry cotton and then by water saturated cotton, at room temperature. In figure 3 are plotted the observed elevations in temperature (above room temperature) of the two outside thermocouples at different rates of flow. The change in the environment from air to water permitted a significant drop in the external junction temperatures indicating greater heat transfer to the environment.

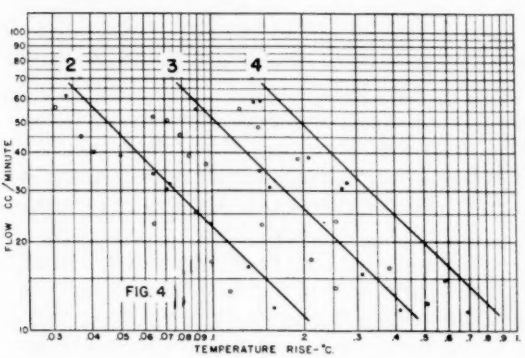
To estimate the total extent of heat loss, the fractional transfer of heat to the fluid passing through the unit and to the surrounding medium was roughly quantitated. The heat generated within the unit was calculated according to Joule's law from the equation $0.239I^2R$ = Cal/Gm/Sec. in which I = 0.2, 0.3 and 0.4, and R = 3.95, 4.15 and 4.28, respectively. The temperature rise of the fluid was determined by an exploring thermocouple fixed in the center of a plastic tube (inside diameter—1 mm.) and located 1 mm. from the tip. This tube was then inserted from the downstream end into the glass tube running through the unit to a point 29 mm. from the downstream edge of the unit. The intervening length of glass tube was packed with glass wool (to insure thorough fluid mixing) and surrounded with layers of tinfoil and dry cotton to minimize heat loss. After calibration of the exploring couple, distilled water was run through the unit at various rates and the corresponding temperature rises observed. The heat loss to the environment was estimated indirectly by subtracting the heat uptake by the fluid from that generated by the heater.

Since Cal/Gm/Sec. at 60 cc./min. is numerically equal to the temperature rise in °C at that flow and the temperature rise of the fluid should vary inversely as the flow, a theoretical temperature for any flow may be plotted. Undoubtedly a small heat loss occurred from the point at which the fluid left the unit to that of recording. However, such losses are negligible compared to those sustained to the external environment of the unit during its operation.

In figure 4 are shown points representing a rough quantitation of the heat transfer to the fluid passing through the unit at different rates of flow and at three applied heating intensities. The extent of heat transfer is represented in terms of temperature elevation above that of the incoming fluid (room tem-

perature). The interval separating the points from the curves (indicating theoretically optimal or 100 per cent transfer) represents the degree of heat loss via the block.

While the heat losses to an environment of quiescent air (dry cotton) are detectable but small, those to water saturated cotton are sizable, averaging 25 to 30 per cent over all ranges. Similarly, an environment of air with 100 per cent water saturation ("moist chamber") in contrast with that of muscle tissue yielded results (points not shown) which were indistinguishable from those obtained above with room air versus water. Such differences could be expected considering the different abilities of these substances to take up heat per unit *volume* (water = 1 Cal/cc./°C rise, air = 0.0003 Cal/cc./°C rise).



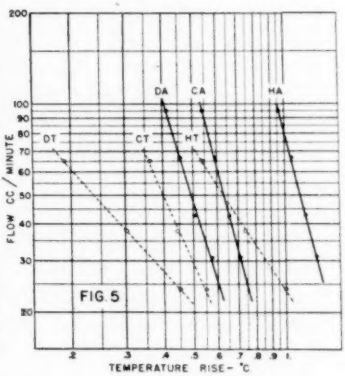


Fig. 4. Graph indicating heat transfer to water perfusing unit in terms of temperature elevation. Curves indicate theoretical 100 per cent transfer at three heating intensitives. (For data see text.) Dots—thermostromuhr surrounded by air (dry cotton). Circles—thermostromuhr surrounded by water saturated cotton. 2, 3, and 4—same as in figure 1. Ordinate and abscissa—same as in figure 1.

Fig. 5. Graph showing temperature elevations of individual thermocouples of thermostromuhr with derived differential temperatures with external environments of air (dry cotton) and of muscle tissue. Heater current—200 MA. *A*, air environment; *T*, tissue environment; *H*, *C* and *D*, same as in figure 1. Ordinate and abscissa—same as in figure 1.

From the foregoing, it would seem likely that the thermocouples lying intimately close to the passing fluid would be particularly affected by change in environment. To investigate this point the special unit for individual couple temperature measurements was placed in a pump system and the temperatures of both couples together with the differential temperatures were determined with the exposed surface of the unit surrounded by dry cotton and then by muscle tissue at room temperature.

As seen in figure 5, the presence of tissue depresses the temperatures of both couples, but by different amounts. Since the new temperature curves do not parallel the controls, the major influence is presumably the depression of the couple temperatures directly, by affording a more rapid exit of heat from the couple areas to the external environment.

Such heat loss may become even more significant when units are applied in animals. A living tissue environment may not always be considered a *fixed* environment as with the use of dead tissue. Blood flow through the tissue may not only increase the total heat loss from the unit, but may selectively affect the area over one thermocouple more than the other (1).

Influences, internally induced. Additional influences affecting the heat gradients at the thermal junctions are the presence of zero flow, back flow and turbulence in the fluid passing through the unit.

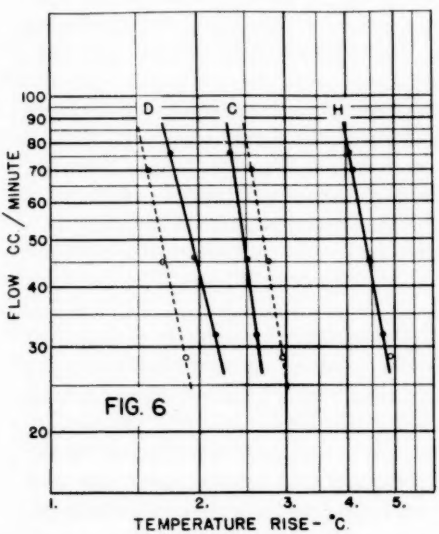
In a previous paper (1) the presence of zero flow in the flow pattern caused a diminution in the galvanometer deflection, as observed in a conventional unit. To quantitate the changes in the individual couple temperatures the special unit in connection with a pump system was used. A motor operated valve permitted interruption and cessation of flow for any desired period of the cycle. Exact measurement of the flow components could be obtained from the record of an orifice plate meter (2) interposed in the line.

The effects of 35 per cent zero flow upon each of the couples separately, together with the net effect exerted upon the differential temperature curve, is presented in figure 6. These values are to be contrasted with the control curves obtained with an uninterrupted flow. The obvious change is the increase in the temperature of the cold junction.

To maintain the same flow per minute when flow ceases (zero flow) during part of each cycle, the velocity of the forward flow of the fluid through the unit must be greater than that which existed during the constant control flow. Under these conditions, the couple temperatures as recorded represent *means* derived from rapidly changing heat gradients. Since the cooling effect of the fluid is extremely small when the flow is zero and is much larger during the necessarily greater forward velocity required to give the same minute flow, the temperature of each couple would be expected to vary with the alternating components of the flow cycle. This was confirmed to the extent of observing 2 to 3 mm. oscillations of the galvanometer deflection synchronous with the interruptions in flow. The ultimate mean temperature of each couple is therefore determined by the final mean position it occupies in the rapidly changing gradients. The mean gradient about the cold junction has undergone the greater shift from the control level. The apparent indifference of the hot junction should not be regarded as indicating its invulnerability to such changes, but merely as evidence of a closer balance of the two opposing influences at that site.

That ordinary calibrations are not applicable to the measurement of flows having periods of zero flow could have been predicted on the basis of the theoretical consideration of Burton (3) and the experimental findings of Herrick, Baldes and Sedgwick (4). Studies of the operation of the Rein thermostromuhr revealed that flows of small magnitude fail to vary with the galvanometric deflection in the relation $GV^N = K$, in which G is the galvanometric deflection, V the flow in cubic centimeters per minute, and N and K are constants for the particular unit. Since Burton points out that the same would essentially hold

for the direct current thermostromuhr, a conventional calibration at higher flows, in which the above relation does exist, is therefore not applicable to flows in which part of each cycle has a rate of flow of a few hundredths cubic centimeter per minute, or less (near zero or zero flow). We concur in the contention that the "thermostromuhr indicates cessation of flow or very small flows by decreasing deflection" not only when such flows are prolonged, but also when either is present in the *flow cycle*.



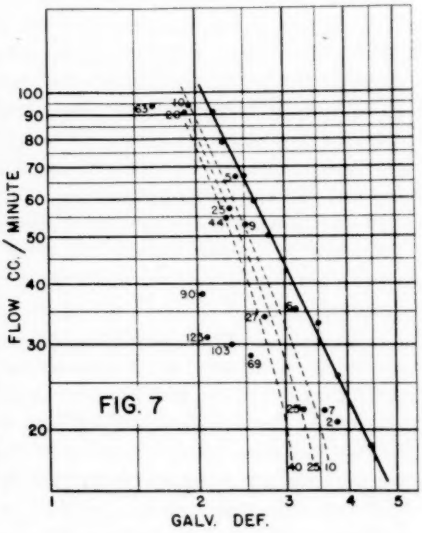


Fig. 6. Curves indicating temperature elevations of individual thermocouples of thermostromuhr with and without presence of zero flow (35 per cent of cycle time) in flow pattern of fluid metered. Heater current—400 MA. Dots—control uninterrupted flow. Circles —35 per cent zero flow in in each cycle. *H*, *C* and *D*—same as in figure 1. Ordinate and abscissa—same as in figure 1.

Fig. 7. Graph showing the effects of periods of back flow in the flow pattern on the galvanometric deflections obtained with a conventional thermostromuhr unit plotted against flows measured in a graduate. Continuous line—calibration with uninterrupted flow, all other conditions remaining the same. For further description see text. Ordinate—flow in cc./min. Abscissa—galvanometric deflection in 100 mm.

The effects produced by the presence of back flow in the flow pattern are not dissimilar to those discussed above. To obtain such a flow pattern the same schema was used as for the study of zero flow, except for the replacement of the stopcock with a short length of resilient rubber tubing. For the production of the back flow the tubing was partially compressed and suddenly released by a motor operated lever arm. The proportion of each cycle occupied by the period of back flow was measured from the record of the orifice plate meter, while the volume of back flow was determined by calculating the volume of "suck-back" into a piece of transparent plastic tubing attached to the peripheral end of the system.

In figure 7 are shown galvanometer readings (differential temperatures) plotted against actual flows with various volumes of back flow per cycle. The positions of these points are to be contrasted with that of the calibration curve obtained in the presence of an uninterrupted flow under the same conditions. The number near each point represents the corresponding volume back flow per cycle in cubic millimeters. Indicated by interrupted lines are the positions to which one would expect the calibration curve to shift in the presence of the volume back flow per cycle noted below each.

Back flows of a volume less than the volume of fluid existing between the heater and the cold junction (9 cu.mm. by measurement) exert a relatively small but definite effect on the calibration curve.

By use of the special unit and with the back flow volume per cycle kept as near to 44 cu.mm. as possible for the readings at three flow levels, the shift of the couple temperatures is very similar in extent to that observed in the presence of 35 per cent zero flow (fig. 6), although the interruption of the forward flow occupies only 10 per cent of the cycle or less. Small galvanometer oscillations were again observed indicating that the couple temperatures were mean values resulting from the alternation of at least two different temperature gradients.

The mechanism determining the gradients would appear to be slightly different in the case of back flow since the fluid not only ceases flowing at two points in the cycle, but the two thermocouples reverse their orientation with respect to the direction of flow for a certain part of the cycle, the "upstream" becoming "downstream" and vice versa. A consideration dealing with the interplay of these rapidly changing dynamic and static states insofar as they determine the temperature gradients around the couples would necessitate investigation beyond the scope of this paper. However, superficially a back flow of fluid carries already heated fluid past the areas from which it received part of its heat (fig. 2). The relative cooling effect upon any given area depends upon the respective temperatures of the area and the fluid going by it. The cold junction area, being at a relatively low temperature because of its originally predominate upstream position is, therefore, particularly affected by the reversal of the warmed fluid stream.

Theoretically, the thermostromuhr, when conventionally calibrated, is incapable of quantitating flow in the presence of back flow. With the Baldes and Herrick or Rein type unit the galvanometer deflection is theoretically zero when flow is zero. With equal amounts of forward flow and back flow the total flow is still zero, deflection zero and the mean flow zero. Hence, any amount of back flow will presumably nullify or neutralize to a definite extent the effects of forward flow, but the recorded empirical "mean" flow is not necessarily a difference, mean or average of the two flow components. A calibration obtained with flow proceeding in one direction only obviously cannot be applied to deflection values obtained under conditions in which the flow is periodically reversing its direction.

In the literature (5, 6, 7) and in public discussion it has been stated or im-

plied that the presence of turbulence in the fluid metered constitutes an important source of error. In anticipation of the possibility that zero flow and back flow effects could result from the coincident production of turbulent flow, a study of the production and effects of turbulence in the thermostromuhr was undertaken.

Turbulence, as defined, is a state of agitation or confusion, and as far as can be learned is incapable of quantitation. It is presumed, however, that when the conduct of a fluid as it passes through a tubular structure deviates from a "streamline" progression, such a state may then be called turbulence.

It is difficult to determine what degree of turbulence may exist in blood flowing through a blood vessel, owing to the relative opacity of the two substances. To obviate this, translucent or transparent tubes of glass, plastic, and rubber perfused with acacia solution (spec. visc. 4.0–4.5) were substituted in these studies. In the acacia solution were suspended extremely fine aluminum flakes (8) in the amount of 0.5 gm./L. Because of the small size and weight of the aluminum particles and the relatively high viscosity of the medium no appreciable settling was noted even if the mixture were left undisturbed for several minutes. With this degree of stability the conduct of the particles as observed in a microscope was assumed to reflect fairly well the conduct of the medium in the immediate vicinity.

First, attempts were made to visualize to what extent turbulence might exist under conditions similar to those in small arteries *in vivo*. A thin, translucent walled rubber tube 2 mm. in diameter was constructed so as to simulate in conformation and elasticity a small tortuous artery (fig. 8-A). The acacia mixture was pumped through this artificial artery so that the pulse waves gave rise to pulsatile expansion, flexion, torsion, and elongation of the tube. As observed through a microscope, no turbulence was noted within the tube with flows up to 100 cc. per minute. Angulation and indentation of this and other tubes of equal bore to the extent shown in figure 8-B, C, D, E, failed to induce visibly turbulent flow beyond or at the deformity.

While turbulence is not readily obtained under such conditions in a tubular structure of this size more abrupt constrictions in the bore (fig. 8-F, G) set up marked degrees of whirling turbulence in the slower moving fluid immediately beyond. (These conditions are not dissimilar to those which may exist during *in vitro* calibration of a unit.)

Having produced a definitely turbulent flow by somewhat unnatural means, the effects upon the thermostromuhr were next considered. A special unit was constructed similar to that used above, but with conventional wiring and with such precautions as to afford maximal transparency through all portions for observation under a microscope. In the upstream end of the glass tube, running through the unit, a plastic ring (similar to that shown in fig. 8-G) was placed 5 mm. from the upstream edge of the unit. During perfusion with the acacia mixture turbulence was seen to exist opposite the area of the cold junction, but no change in the galvanometer deflection from the control reading was observed. Moving the ring to positions 3, 2 and 1 mm. from the edge of

the unit permitted extension of the turbulence further into the unit resulting in the respective shifts in the deflection from the control calibration shown in figure 9. The appearance and extent of the turbulence at several flow levels has been diagrammed for one series (fig. 8–9, H, I, J, K).

Attempts to obtain similar effects under somewhat less abnormal conditions failed. A conventional unit was applied to a snugly fitting artery segment and

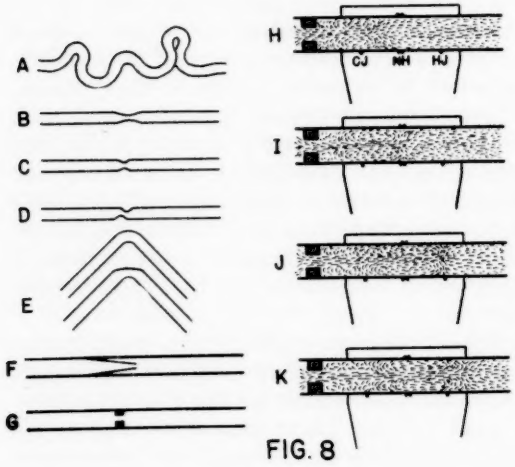


FIG. 8

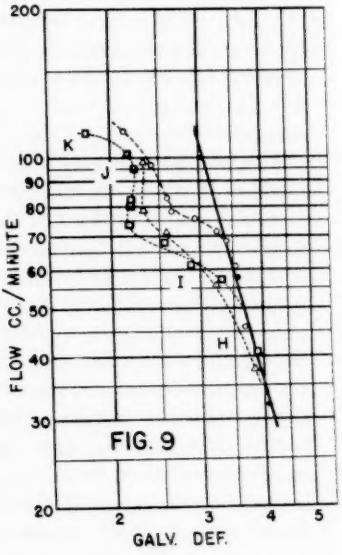


FIG. 9

Fig. 8. *A*, *B*, *C*, *D* and *E*, diagrams illustrating conformation and deformities of tubes through which the passage of fluid under conditions described in text gave rise to no observable turbulent flow. *F*, *G*, diagrams illustrating abrupt changes in bore of tubes by means of which decidedly turbulent flow was produced. *H*, *I*, *J* and *K*, appearance of flow showing nature of turbulence and its relation to the thermocouples and heater elements as observed through a transparent thermostromuhr unit. Letters correspond to flow levels denoted by same letters in figure 9 in relation to the curve indicated by squares. *CJ*, cold junction; *HJ*, hot junction; *NH*, heater.

Fig. 9. Graph showing effects of demonstrable turbulence within thermostromuhr unit upon the galvanometric deflection. Dots and uninterrupted line—calibration in the absence of turbulent flow. Circles—constricting ring placed 3 mm. from upstream edge of unit. Squares—ring placed 2 mm. away. *H*, *I*, *J*, and *K* indicate points on same curve at which turbulent patterns shown in figure 8 were observed. Triangles—ring placed 1 mm. from upstream edge of unit. Ordinate and abscissa—same as in figure 7.

a calibration obtained. Angulation of the artery at the point of entrance into the unit 90° or less failed to shift the galvanometric deflection at any flow level, provided precautions were taken to prevent variation in *artery contact* through such manipulation. Without these precautions such angulation at the downstream end alone induced marked effects, thereby dismissing turbulence *within* the unit as the causative factor.

Lastly, the ring was removed and the pump system re-arranged so as to deliver flows with either zero flow or back flow through the unit. Direct observation revealed nothing suggestive of turbulence in any part of the cycle, with wide variations in the amount of zero flow or back flow.

It is believed that the numerical data presented in this paper are applicable to the factors they delimit insofar as they indicate *qualitative* and *directional* changes. Since any quantitative data are applicable only to a given unit under given conditions, the *quantitative* significance of the data in the foregoing experiments is therefore specific only for the unit and conditions so described.

SUMMARY

In the application of the direct current thermostromuhr of the Baldes and Herrick type to the measurement of blood flow, several factors other than the rate of flow were found to influence the empirical flow readings, namely, 1, changes in the environment of the thermostromuhr unit, 2, the presence of zero flow, and 3, back flow in the flow pattern (1).

The mechanism of operation of the thermostromuhr was examined from the standpoint of qualitative changes produced in the heat gradients about the thermocouples. Fundamentally, the thermocouples, heated primarily by direct conduction through the block from the heater area are *cooled* by the fluid passing through the unit since their individual temperatures are found to be greater than the corresponding adjacent fluid passing by each. The extent to which each couple is thereby heated and cooled in relation to flow determines the differential temperature and therefore the galvanometer deflection at any flow. The latter relation constitutes the so-called calibration.

Changing the external environment of a unit was found to alter the thermocouple temperatures through changes in the rate of heat loss to the environment.

It is concluded that any calibration of a thermostromuhr unit is applicable to the operation of the unit only under environmental conditions identical to those under which the calibration is obtained.

This also applies to internal environmental changes where the presence of zero flow or back flow in the flow pattern of the fluid metered renders worthless the empirical mean flow values as read from a conventional calibration. Such apparent incompetence of the thermostromuhr is the direct result of an unwarranted application of an inappropriate calibration rather than evidence of an inherent physical incapability.

Turbulent flow and its influence upon the operation of the thermostromuhr were examined under several abnormal conditions. It is believed that the extent to which turbulence may naturally exist in a small, healthy blood vessel is insufficient to produce effects worth consideration in the face of more demonstrable influences which impair the accuracy of the instrument. Indeed, if turbulence of appreciable magnitude does at times occur in the arteries of normal animals, there is even less possibility of detection or control than exists in the presence of other various influential variables.

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THE HOMEOSTATIC RÔLE OF A RENAL HUMORAL MECHANISM IN HEMORRHAGE AND SHOCK[1]

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Since exaggerations of normal processes often result in pathological conditions, it would be surprising if the humoral mechanism involved in renal hypertension did not have a physiological counterpart. It appeared likely that this mechanism would be evoked by various emergencies altering renal circulation, and therefore constitute an important homeostatic factor.

Wakerlin and Chobot (1939) and House and Wakerlin (1941a, 1941b) found no evidence for a specific rôle of the kidney in the regulation of normal blood pressure. In a preliminary abstract Hamilton and Collins (1941) found that the kidneys are responsible for a pressor material which aids in the maintenance of arterial blood pressure during hemorrhage. The present paper is an amplification and extension of these results. At about the same time Sapirstein, Ogden and Southard (1941) found that blood taken after hemorrhage had a renin-like action on guinea-pig ileum; the responsibility of the kidney for the development of this property was not established.

METHODS. Fifty-seven experiments were performed on mongrel dogs. All animals were anesthetized, usually with alpha-chloralose given intravenously as a 1 per cent solution (in Locke's fluid without glucose). The initial dose was 10 cc. per kgm. of body weight, and more was given when necessary in certain types of experiments. Combined morphine-chloralose anesthesia was used occasionally. Recipients, used as test animals, were anesthetized as above or with sodium pentobarbital. Mean arterial blood pressure was recorded from the carotid artery with a mercury manometer. All procedures involving the renal vessels, kidneys or adrenals were done through the retroperitoneal approach. Heparin was added to blood used for tests or reinjection. Blood was tested for pressor activity by observation of the changes in blood pressure resulting from injection into a small, nephrectomized dog. The blood was introduced at body temperature from a burette into the left femoral vein, while the same amount was withdrawn synchronously from the right femoral artery; changes in the blood volume of the recipient were thus avoided. Renal venous blood was obtained from the donor by means of a bent hypodermic needle attached to a syringe. Great care was taken to avoid any renal congestion or ischemia. Blood was also taken from the femoral artery and in a few cases from the inferior vena cava. The samples were tested shortly after they were taken.

RESULTS. 1. *Studies of the pressor activity of blood in hemorrhage.* (Table 1.) Samples of blood taken before hemorrhage from either the renal vein or

[1] This investigation was aided by a grant from the American Philosophical Society.

TABLE 1

Pressor responses from the blood of animals subjected to hemorrhage

All tests were made in nephrectomized recipients. The hemorrhages were not made at a constant rate; the time between sampling and the end of hemorrhage varied; different amounts of chloralose involving variations in fluid volume were given before hemorrhage. R, A and CV signify that the samples were taken from the renal vein, femoral artery, and inferior vena cava.

EXP. NO.	CONTROL BLD.		TOTAL BLOOD LOSS	AFTER HEMORRHAGE			
	Vol. of sample	Rise		Vol. of sample	B.P. when taken	Rise	Remarks
			A. Intact animals				
	cc.	*mm. Hg*	*cc. per kgm.*	*cc.*	*mm. Hg*	*mm. Hg*	
1	100R	5	45	53R	50	16	B.P. of donor rising
2	46A	2	38	36R	50	24	
3	45A	3	38	40R	50	15	
4	58R	4	34	24R	60	62	Manipulative renal ischemia
5	60R	4	39	47R	100	20	B.P. of donor rising
			43	47A		9	
6	44R	0	40	32R	90	28	B.P. of donor rising
			43	32A		13	
7	42R	6	36	42R	56	18	B.P. of donor falling
			41	42A		12	
8	42R	0	34	42R	40	40	B.P. of donor falling
	42A	0	34	42A	40	40	
			B. Renal denervation				
9			38	26R	53	26	B.P. of donor falling
			38	44A		22	
			C. Adrenalectomized dogs				
10	23R	8	17	13R	28	10	Donor died soon after sample
11	42R	10	35	42R	60	19	B.P. of donor falling
			38	42A		14	
12	42R	Fall	25	20R	36	31	Donor died as sample was taken
13	42R	0	20	42R	50	17	B.P. of donor falling
	42A	0	25	42A		11	
14	42R	0	19	42R	54	24	
	42A	0	19	42A		20	
15	42A	2	10.9	42R	44	12	
			10.9	42A	44	9	
			16.3	42R	32	23	
			16.3	42A	32	17	
			21.3	30CV		21	Dog died as sample was taken

femoral artery of the donor gave insignificant pressor responses. The results following hemorrhage will be considered separately in the various types of experiments.

Eight intact animals were first studied (table 1, A). About one hour after hemorrhage (34–45 cc. per kgm. of body weight) bloods from the renal vein and femoral artery gave rises of 15 to 40 and 9 to 40 mm. Hg respectively. In many cases the reactions were renin-like, and persisted 10 to 30 minutes. In other cases the elevations of blood pressure lasted 3 to 10 minutes, and approximated, therefore, the duration of angiotonin responses. This difference in the duration of the responses was not related to the magnitude of the rise. In two experiments (5 and 6), where the blood pressures were relatively high at the time of sampling (100 and 90 mm. Hg), blood from the renal vein possessed a much greater activity than arterial blood. In the other experiments of this group and also in those performed on adrenalectomized dogs (table 1, C), the blood pressures were lower and the renal arterio-venous differences less. In one intact animal (expt. 8), in which the blood pressure was continuously falling, samples taken at 40 mm. Hg showed no arterio-venous difference.

In one experiment (table 1, B) with renal denervation hemorrhage resulted in good pressor responses. This finding indicates that the production of pressor material does not depend entirely on connections with the central nervous system.

The renal arterio-venous differences obtained suggest that the source of the pressor material is the kidney. The following experiments give conclusive proof for a renal origin. In six acutely adrenalectomized dogs hemorrhage uniformly resulted in the appearance of pressor material (table 1, C). In three additional experiments both kidneys and adrenals were removed, and several arterial samples were tested during the progress of the hemorrhage. Pressor material failed to appear even with lethal hemorrhage. Thus in the adrenalectomized animal the kidney is essential for pressor activity. The contribution which the adrenals may make in similar hypotension was determined in three nephrectomized animals with intact adrenals. In two cases blood from the vena cava near the orifices of the phrenico-abdominal veins gave epinephrine-like responses of 18 (expt. 20) and 75 (expt. 21) mm. Hg. The arterial sample of experiment 21 taken immediately before death gave a transitory (1 min.) epinephrine-like rise of 13 mm. Hg, and was the only arterial blood which showed pressor activity.

2. *Effect of the kidneys on the ability to maintain arterial blood pressure in hemorrhage.* If this renal humoral mechanism is important in homeostasis, the ability to maintain blood pressure in hemorrhage should be impaired in the absence of renal circulation. Observations were made on twenty-four dogs, anesthetized with chloralose. Since it was necessary to avoid inequalities in fluid administration, only the initial dose of chloralose was given.

Observations on the blood loss necessary to lower the blood pressure to 30 mm. Hg were made on eight dogs with intact renal circulation and on four nephrectomized dogs used primarily for a study of renin tachyphylaxis. Four of the animals with intact kidneys were subjected to sham-nephrectomy, consisting of bilateral renal exposure and manipulations in the wound. The hemorrhage consisted of repeated bleedings. The blood pressures of the nephrectomized

animals were lowered to 30 mm. Hg in $1\frac{1}{6}$ hours, while in the intact animals the hemorrhage extended over 2 hours although there was a greater blood loss at a faster rate. The blood losses in cubic centimeters per kilogram of body weight were as follows: group with intact kidneys and no sham-nephrectomy—46.0, 44.2, 58.6, 45.4 (average—48.6); sham-nephrectomized group—44.2, 50.3, 45.3, 51.1 (average—47.7); nephrectomized group—42.8, 23.6, 37.6, 36.6 (average—35.2).

More carefully controlled studies were made on twelve additional dogs (4.5–11.1 kgm.). In six animals the renal vessels and ureters were tied with as little disturbance as possible to the nerves, and the kidneys were left *in situ*. Identical operations were performed in the remaining six dogs except that renal circulation was left intact and ureters were not tied. The animals were then bled; the rate and total amount of blood loss were rigidly constant (fig. 1). The falls in blood pressure occurred earlier and were greater in the dogs with interrupted renal circulation. Shortly after the last bleeding the average difference in blood pressure of the two groups was 40 mm. Hg. Half of the animals without renal circulation died shortly after completion of hemorrhage, while no fatalities occurred in the other group. Since the procedures involved minimal disturbance to the adrenals, it is unlikely that injury of these organs played a rôle in these differences.

Thus an intact renal circulation aids in the maintenance of arterial blood pressure in hemorrhage, and thereby constitutes an important compensatory mechanism.

3. *Tachyphylaxis in extreme hemorrhage.* Since repeated injections of renin result in tachyphylaxis, it appeared possible that the renal compensatory mechanism might become crippled in severe, prolonged hypotension. Experiments were therefore performed to test the hypothesis that severely bled animals should become tachyphylactic to injected renin because their kidneys had liberated large quantities of this material.

The blood pressures of ten dogs with intact renal circulation and six nephrectomized animals were lowered and then maintained by hemorrhage at shock levels (60–30 mm. Hg) for about $1\frac{3}{4}$ hours. The pressures of the intact and of the nephrectomized dogs were maintained at about 30 mm. Hg for 0 to 40 and 30 to 65 minutes respectively. Test doses of epinephrine (0.2 cc. of 1:5000) and renin[2] (0.1 cc.) were given intravenously before hemorrhage and after the period of hypotension. Blood was returned when necessary to prevent death. In most cases blood or Locke's fluid was administered before the final injections, since renin was not given until the animal was capable of responding vigorously to epinephrine. After hemorrhage the responses to renin of dogs with intact renal circulation were either absent or markedly reduced, while in the nephrectomized animals the average response was slightly greater than its control. Figure 2 is a composite diagram illustrating the findings. The results of only eight of the sixteen dogs are included since sham-nephrectomies were not done in some and in others renal denervation was performed. However, the results

[2] We are greatly indebted to Dr. Irvine H. Page for supplying the renin.

on the eight excluded dogs coincided with the others. The difference in the response to renin after hemorrhage of dogs with and without renal circulation occurred in spite of the following conditions: 1, the average response to epinephrine was about equal in both groups; 2, pitressin gave vigorous responses in both groups; 3, the intact animals had a higher average blood pressure just before injection of renin; 4, the nephrectomized animals had been subjected to a longer and more severe hypotension.

The occurrence of increased responses to renin following the bleeding in some of the nephrectomized animals is not surprising. Wakerlin and Chobot (1939) have reported that nephrectomy increases the dog's reactivity to renin even in 1 to 3 hours. We have confirmed this observation in three dogs anesthetized with chloralose. While this potentiation complicates the interpretation of our experiments, the findings suggest that the intact animal subjected to severe

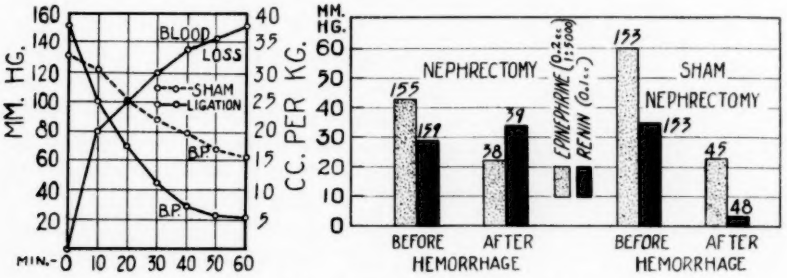

Fig. 1. Composite curves of the blood pressures of 6 dogs in which the renal vessels and ureters were tied and of 6 dogs with corresponding sham-procedures.

Fig. 2. Diagram showing the average change in blood pressure of 4 nephrectomized and 4 sham-nephrectomized dogs in response to injections of epinephrine and of renin before and after severe, prolonged hypotension produced by hemorrhage. The figure at the top of each rectangle is the average, mean blood pressure just before the injection.

hypotension for some time fails to respond well to renin because his kidneys have been liberating relatively large quantities of this material. It is interesting that under similar conditions of hypotension small renal arterio-venous differences in pressor activity were found.

Three intact animals with a less severe hemorrhage, 38 cc. per kgm. of body weight (included in fig. 1), were tested with renin before hemorrhage and one hour after its completion. In two animals the blood pressures fell to 78 and 54 mm. Hg at the end of the bleeding, and then climbed steadily. Renin was injected when the pressures were 113 and 89 mm. Hg respectively, and the responses were undiminished. Thus when the blood pressure was relatively high and compensation was occurring, tachyphylaxis was not evident. In the third dog the blood pressure fell to 54 mm. Hg, returned to 94, and then fell slowly to 78 mm. Hg. The response to renin was only 12 mm. Hg as compared to the control response of 34 mm. Hg.

4. *Histamine.* The pressor activity of the blood of three intact and three

acutely adrenalectomized dogs was studied. In order to maintain the blood pressure at shock levels (usually between 30 and 60 mm. Hg) histamine was injected in divided doses (one exception) into the external jugular vein. The intact animals required a total of 1.06 to 1.59 mgm. per kgm. body weight, while only 0.147 to 0.417 mgm. per kgm. was required in the adrenalectomized dogs. Blood removed in sampling was replaced with blood from the recipient.

TABLE 2

Pressor responses from the blood of animals during histamine hypotension

All tests were made in nephrectomized recipients. Repeated doses of histamine were often given to maintain hypotension. Source of sample: A = femoral artery blood, R = renal vein blood.

EXP. NO.	CONTROL BLD.		WT. OF DOG	TOTAL HISTAMINE	AFTER HISTAMINE				REMARKS
	Vol. of sample	Rise			Length of hypotension	Vol. of sample	B.P. when taken	Rise	
					Intact dogs				
	cc.	*mm. Hg*	*kgm.*	*mgm.*	*hrs.:min.*	*cc.*	*mm. Hg*	*mm. Hg*	
1	42R	0	7.7	2.6	0 : 56	42A	50	10	
				7.2	1 : 40	42A	30	14	
				9.4	2 : 21	42A	46	18	
				9.4	2 : 28	42R	50	30	
2	42A	0	8.0	10.5	0 : 35	42A	34	−20	Bloody diarrhea
				10.5	0 : 50	42A	56	8	Preliminary fall of 8
				10.5	1 : 5	42R	74	10	
				12.7	1 : 52	42A	40	14	
				12.7	2 : 10	42R	60	14	
3	42R	0	14.2	7.5	0 : 30	42A	44	12	One kidney had been removed
	42A	0		7.5	0 : 45	42R	54	22	
				15.1	1 : 30	42A	20	22	
					Adrenalectomized dogs				
4	42R	0	7.2	3.0	1 : 00	42A	32	14	
	42A	0		3.0	1 : 35	42R	36	18	
5	42R	0	11.2	3.0	0 : 33	42A	26	16	
	42A	0		4.0	1 : 5	42A	36	22	
				4.0	1 : 25	42R	54	30	
6	42A	0	7.5	1.0	0 : 12	42A	44	8	
				1.1	0 : 50	42A	38	10	
				1.1	1 : 14	42R	36	30	
				1.1	2 : 23	42A	34	10	

The production of pressor material occurred in all cases, and was evident in arterial blood as early as ½ hour after the initial dose of histamine (table 2). In experiments where successive arterial samples were taken it was noted that the pressor activity increased with the duration of the hypotension. Although the hypotension was equivalent in degree and length to that of severely bled animals, large renal arterio-venous differences were found in about half of these

experiments. A fall of blood pressure, presumably due to histamine, was obtained from two early blood samples of a dog with a bloody diarrhea (expt. 2). Otherwise the presence of histamine was not detected in recipients even, in one case, as early as eight minutes after a histamine injection of 2.3 mgm. into the donor. Finally the renal source of the pressor material was established in two of the adrenalectomized animals; removal of their kidneys resulted in the disappearance of pressor activity from arterial blood.

5. *Adrenalectomy.* In the present investigation fifteen dogs were adrenalectomized primarily to eliminate the liberation of epinephrine. However, during the 4 or 5 hours following adrenalectomy additional interesting and surprising findings were observed which warrant reporting.

Since the removal of both adrenals at one operation often causes "shock," special care was taken to select dogs in excellent condition. After operation the dogs were left undisturbed for about 2 hours before control renal and femoral samples were taken. Control bloods of three of these animals caused marked falls in the blood pressure of the recipients. Donor 1 had a pressure of 86 mm. Hg. after withdrawal of the samples. When the renal sample was injected into a nephrectomized recipient, the blood pressure fell 130 mm. Hg, and remained low for a long time. However, the femoral sample produced no depression after standing at 37°C. for $1\frac{1}{6}$ hours, but the donor's blood still showed depressor activity (fall of 39 mm. Hg) $3\frac{2}{3}$ hours after adrenalectomy. Donor 2 had a blood pressure of 94 mm. Hg after withdrawal of control samples. The recipient's pressure fell 67 mm. Hg after injection of the renal sample, and remained depressed. A later sample, taken $2\frac{5}{12}$ hours after adrenalectomy, showed no depressor activity. When a total of 25 cc. of blood per kgm. of body weight had been removed from this donor, a 20 cc. sample of renal vein blood, obtained $4\frac{1}{3}$ hours after adrenalectomy, produced a *rise* of 31 mm. Hg in the recipient's blood pressure. Withdrawal of this sample caused death of the animal. A control arterial sample of donor 3 produced a fall of 38 mm. Hg in the recipient, and another sample taken $2\frac{5}{6}$ hours after operation still showed depressor activity. Cross agglutination tests were made in this experiment, and the bloods were compatible. It is interesting that small amounts of blood (42 cc.) from donors whose blood pressures were above shock levels gave extreme falls in nephrectomized recipients.

On the assumption that the liberation of depressor material resulted from the surgical technique, the remaining adrenalectomies were performed under deeper anesthesia, the phrenico-abdominal veins were first ligated, and the other vessels, nerves, and tissues were severed by electro-coagulation instead of by ligation and cutting. The blood of ten dogs adrenalectomized by this improved technique showed no depressor activity. In spite of blood pressures within the normal range, adrenalectomized dogs were very sensitive to hemorrhage and to histamine. The withdrawal of 10 cc. of blood per kgm. gave sharp falls in blood pressure with little tendency toward recovery, while most intact animals showed little change after the loss of 20 cc. per kgm. at one bleeding. Regardless of the degree of hemorrhage, when the blood pressures of the adrenalectomized

animals were reduced to a low level, the renal arterio-venous differences in pressor material were small. Finally, adrenalectomized dogs, as compared with intact animals, required only about $\frac{1}{5}$ as much histamine to maintain their blood pressures between 30 and 60 mm. Hg. Since the histamine was given $2\frac{1}{4}$ to $2\frac{1}{2}$ hours after the adrenalectomy, this marked sensitivity developed within this period.

Discussion. Our experiments demonstrate that the kidney is responsible for the development of pressor activity in blood both after hemorrhage and after histamine injection. These findings and the report by Taquini (1938) that vasoconstrictor properties appear in renal vein blood after asphyxia suggest that the renal humoral mechanism may be involved in a wide variety of emergencies. The kidney is thus an endocrine gland, and its internal secretion is liberated in various types of stress. That the kidney aids in homeostasis is shown by the observation that absence of renal circulation impairs the ability to maintain blood pressure in hemorrhage.

We have made no consistent attempt to determine threshold values for time or degree of hypotension necessary to evoke the renal mechanism. Pressor activity was noted in arterial blood after $\frac{1}{2}$ hour of hypotension, but considerable pressor material might have been produced before it could be detected in arterial blood by our method. The experiments concerning the effect of an intact renal circulation on the ability to maintain blood pressure suggest that the renal mechanism may operate after a relatively mild renal stimulus. Animals without renal circulation showed a marked drop of blood pressure shortly after the initial bleeding of 20 cc. per kgm. of body weight, while the fall was relatively small in intact dogs. This early involvement of the renal humoral mechanism is consistent with data from studies on renal hypertension. Verney and Vogt (1938), Braun-Menendez and Fasciolo (1939), Friedman, Seltzer and Sampson (1940–41) and others have reported that only a short period of partial constriction of the renal artery (in some instances a few minutes) is necessary before the blood pressure of the animal rises or vasoconstrictor material can be detected in renal vein blood.

Our findings on tachyphylaxis and the observation that blood after hemorrhage has a renin-like action on ileum (Sapirstein, Ogden and Southard, 1941) suggest but do not prove that the humoral mechanism involved in our experiments is the renin-angiotonin system. The nature of this system renders it susceptible to factors affecting either the liberation of renin or the availability of renin-activator. The liberation of renin would be determined by the degree and duration of the change in renal circulation. Both epinephrine and the renal vasomotor nerves are capable of affecting renal circulation, and may thus influence renin liberation. While adrenalectomy or renal denervation did not prevent the eventual development of pressor activity as a result of hypotension, we believe that these procedures may have a quantitative effect.

Continuous infusions of appropriate amounts of renin produce an excess of renin in the blood and a diminution of renin-activator, followed by tachyphylaxis and disappearance of the initial pressor effect (Page, 1939). In our experi-

ments on hemorrhage, tests in nephrectomized recipients demonstrated the continuous presence of renin-like material in the blood of donor animals subjected to severe, prolonged hypotension; animals under this condition developed tachyphylaxis to injected renin. Exhaustion of renin-activator would be accelerated by the loss of blood containing this substance. Furthermore, the pressor activity of arterial blood approached that of renal vein blood. However, tachyphylaxis was not evident and large renal arterio-venous differences were observed when the blood pressure was well above the critical level after hemorrhage. Under this condition the renin-angiotonin system is presumably operating, since large renal arterio-venous differences and absence of tachyphylaxis were observed by Page (1940, 1941) in experimental renal hypertension. In the histamine experiments, large renal arterio-venous differences were still present in about half of the animals after an hour or more of similar hypotension. This finding may be explained by smaller loss of renin-activator; there was no blood loss, and blood removed in sampling was replaced by blood rich in activator (i.e., from a nephrectomized animal).

SUMMARY

The pressor activity of arterial and renal vein blood was studied by determining the change in blood pressure resulting from injection into a nephrectomized recipient. Control samples did not possess significant pressor activity. After hemorrhage pressor properties consistently appeared in blood from normal dogs, from adrenalectomized dogs, and from one animal with denervated kidneys. The pressor responses in the recipient were often renin-like, persisting 10 to 30 minutes. Other reactions of equal magnitude were shorter, their duration approaching that of responses to angiotonin. Similar observations were made on dogs injected with sufficient histamine to lower their blood pressures to shock levels. That the kidney was responsible for the development of pressor activity was indicated by two findings: 1, renal vein blood usually gave greater responses than the corresponding arterial blood; 2, pressor reactions were obtained from the blood of adrenalectomized dogs, but not from dogs with both kidneys and adrenals removed.

The absence of renal circulation impaired the ability of dogs to maintain arterial blood pressure in hemorrhage.

The above findings indicate that either hemorrhage or histamine evokes a humoral mechanism in the kidney and that this mechanism aids in the maintenance of arterial blood pressure.

Intact animals whose blood pressures had been maintained by hemorrhage at shock levels for about $1\frac{3}{4}$ hours reacted poorly or not at all to injected renin. A group of nephrectomized dogs under similar conditions gave an average response to renin which was slightly greater than the response before hemorrhage. These findings suggest that the kidneys may liberate enough renin to cause tachyphylaxis and thus disable their own compensatory mechanism. Under similar circumstances responses from arterial blood closely approached those from renal vein blood. With less severe hypotension and evident com-

pensation, tachyphylaxis to renin was not observed, and large renal arteriovenous differences were present.

Marked falls of blood pressure were produced in acutely adrenalectomized dogs by relatively mild hemorrhage or small histamine dosage. This increased sensitivity was evident $2\frac{1}{4}$ hours following adrenalectomy.

Falls of blood pressure to shock levels occurred in the recipients upon injection of control blood of 3 dogs, acutely adrenalectomized by ligation and cutting of the nerves and vessels instead of by electro-coagulation.

We wish to express our appreciation for the valuable technical assistance of A. Sokalchuk.

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THE EFFECT OF A CREAM MEAL ON THE ACIDITY AND NEUTRALIZING ABILITY OF THE CONTENTS OF THE DUODENAL BULB IN NORMAL DOGS[1]

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The inhibitory effect of fat on gastric secretion and motility has been well established and the mechanism of this action has been the subject of numerous investigations (9). Little attention, however, has been given to the effect of fat on the reaction in the duodenum (7, 8, 11), especially as regards the acidity changes in this area during the period of active digestion of the fat. Even less is known of the changes in the reaction and neutralizing ability of the contents of the duodenal bulb following the use of fat. The latter is of some practical importance inasmuch as fats have been advocated as useful agents in the management of patients with duodenal ulcer primarily because of their gastric inhibitory action.

This study was undertaken for the purpose of supplying some knowledge of the effects of fat on the acidity and neutralizing ability of the contents of the first part of the duodenum. It was hoped that by comparison with the action of other foodstuffs studied in a similar manner (2, 4) some conclusions could be drawn concerning the influence of the type of food undergoing digestion on the acidity of the duodenal contents. It was further hoped that such a comparison would help to determine whether or not there is a close correlation between the acidity of the gastric and the duodenal contents.

METHOD. Dogs, prepared with cannulated gastric and duodenal fistulas (10, 13), were trained and maintained as described previously (2). As in the previous study, pH and free and total acidity were measured on fractional samples removed simultaneously from the pyloric antrum and the duodenal bulb at 10 minute intervals for ½ hour in the fasting state and for 2½ hours after the beginning of the meal and at ½ hour intervals for an additional hour. The excess neutralizing ability of the duodenal contents was likewise estimated as before.

As a representative fat regular table cream (20 per cent fat) was selected. This was given in 500 cc. quantities so as to duplicate the amount of beef extract solution utilized in another but similar study (4). The cream was fed to all the dogs with the exception of one dog who refused to drink the entire amount, requiring introduction of the remainder through the gastric instillation tube.

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[2] Ross V. Patterson Fellow in Gastroenterology.

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Twenty-three experiments were performed on 5 dogs. In all 2886 different observations were made consisting of 874 determinations of pH, 804 measurements of free acid, 803 measurements of total acidity and 405 measurements of the excess neutralizing ability of the duodenal contents. In expressing the results the 4 specimens collected in the fasting state were averaged to obtain a single fasting value.

RESULTS. *Acidity in pH units.* (Fig. 1.) The average acidity as expressed in terms of pH both in the stomach and in the duodenum was decidedly less than that following a carbohydrate (2) or a protein-histamine meal (4). Not only was the average gastric pH higher but the range of pH values was wider than that encountered after the other meals.

In the duodenal bulb especially the decrease in hydrogen ion concentration was very striking. The average post-meal pH value here was roughly a full unit greater than that after the meat extract-histamine meal and $\frac{3}{4}$ of a unit greater than the average pH following the Ewald meal. The comparative depression of duodenal acidity is further emphasized by the fact that only 1.1 per cent of all the post-cream meal specimens were positive for free acid (pH 3.5 or less) (3), whereas 16.4 per cent of all the duodenal post-Ewald meal samples and 26.5 per cent of all those following the Liebig's extract-histamine stimulation had a pH of 3.5 or less.

Lack of correlation between gastric and duodenal acidity is again seen in the more or less stabilized pH in the duodenum at the same time that the stomach contents display a rising hydrogen ion concentration.

The total duration of the post-meal observation period was $3\frac{1}{2}$ hours. Our failure to note any secondary stimulation of gastric secretory activity such as described by Babkin (1) after the feeding of fat along with other foods is perhaps due to the fact that our observations were not continued long enough.

Free acid. (Fig. 2.) As was to be expected, the gastric free acid values were decidedly lower than following other types of meals studied previously (2, 4). Not only was the average value less but the range of all the readings obtained was only half that seen with bread and water or meat extract-histamine stimulation. Illustrative of the previously noted lack of uniform correlation between the several indexes of gastric acidity (2, 4) is the relatively stable value for average free acid as compared with the steady increase in the average hydrogen ion concentration.

The free acid values of the contents of the first part of the duodenum afford arresting evidence of the marked contribution which cream makes to the neutralizing power of the contents of this region. Although electrometric pH measurements showed the presence of free acid in a small percentage of the samples, the indicator method failed to reveal free acid in any of the post-meal duodenal samples (3). This is in contrast with the demonstration by the same method of free acid in 2.6 per cent of the post-Ewald and 13.1 per cent of the post-meat extract-histamine specimens respectively. It must be admitted, however, that the recognition of the presence of free acid through the use of Toepfer's reagent in duodenal samples containing cream is difficult and probably

erroneous readings were made in some instances even though we attempted to remove the fat by centrifugalization.

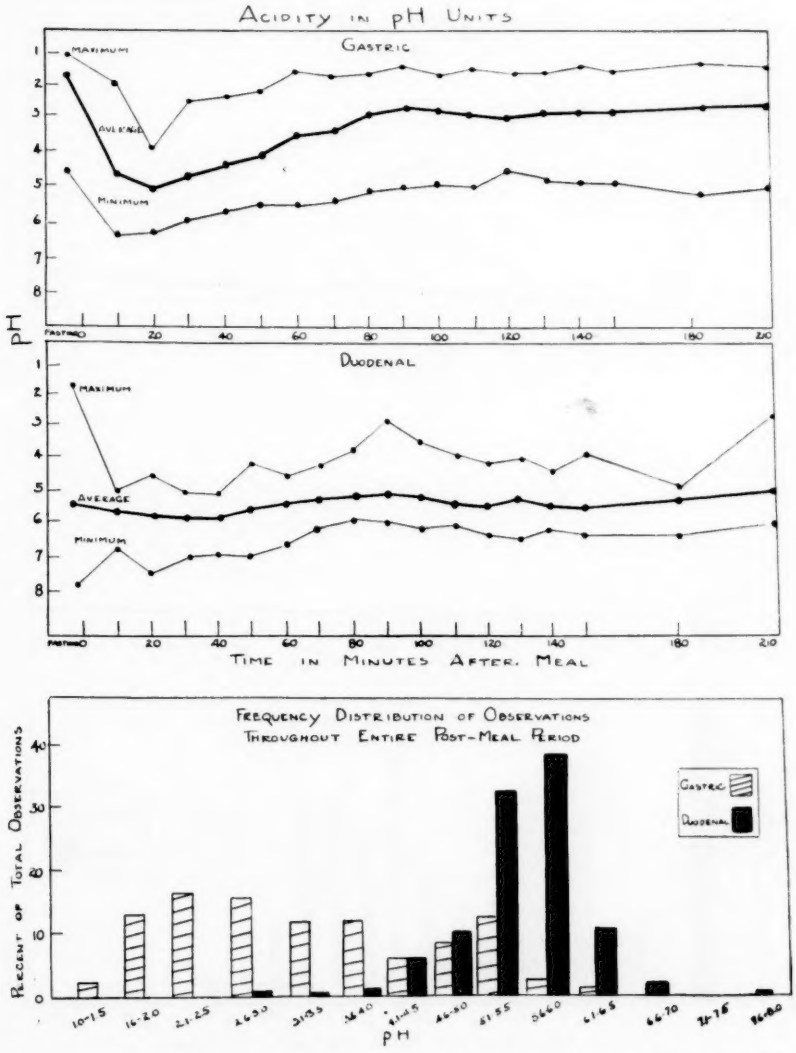

Fig. 1. Acidity in pH units of samples collected simultaneously from just above and just below the pylorus.

It might be well to point out what others have previously remarked on (5), namely, the absence of free acid does not bespeak neutralization in the chemical sense. This is illustrated by the fact that the average pH of the duodenal con-

tents never exceeded the neutral point of 7.0 despite the persistent anacidity as measured by Toepfer's reagent.

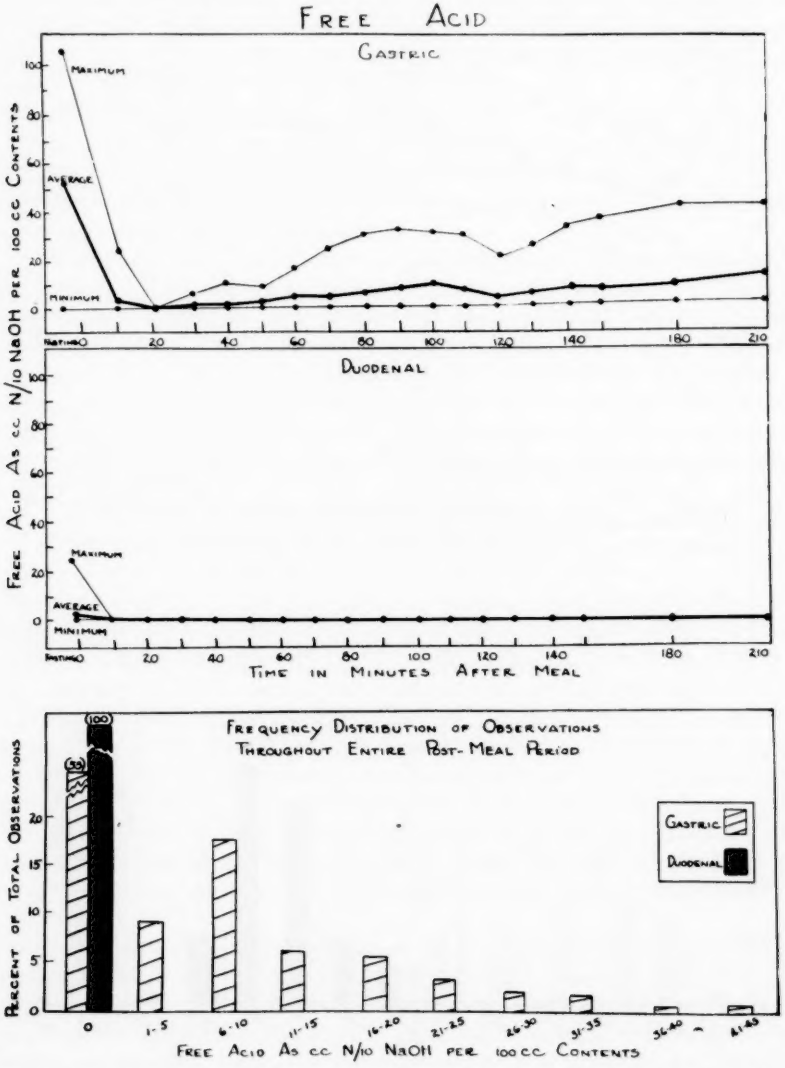

Fig. 2. Free acid as determined on samples collected simultaneously from just above and just below the pylorus.

Total acidity. (Fig. 3.) Again as might be expected the average total titratable acidity of the gastric contents is less following the exhibition of fat than after the use of the other foodstuffs. In the first part of the duodenum, how-

ever, in spite of the higher pH values the average total acidity as found by titration is about equal to that found after meat extract-histamine stimulation

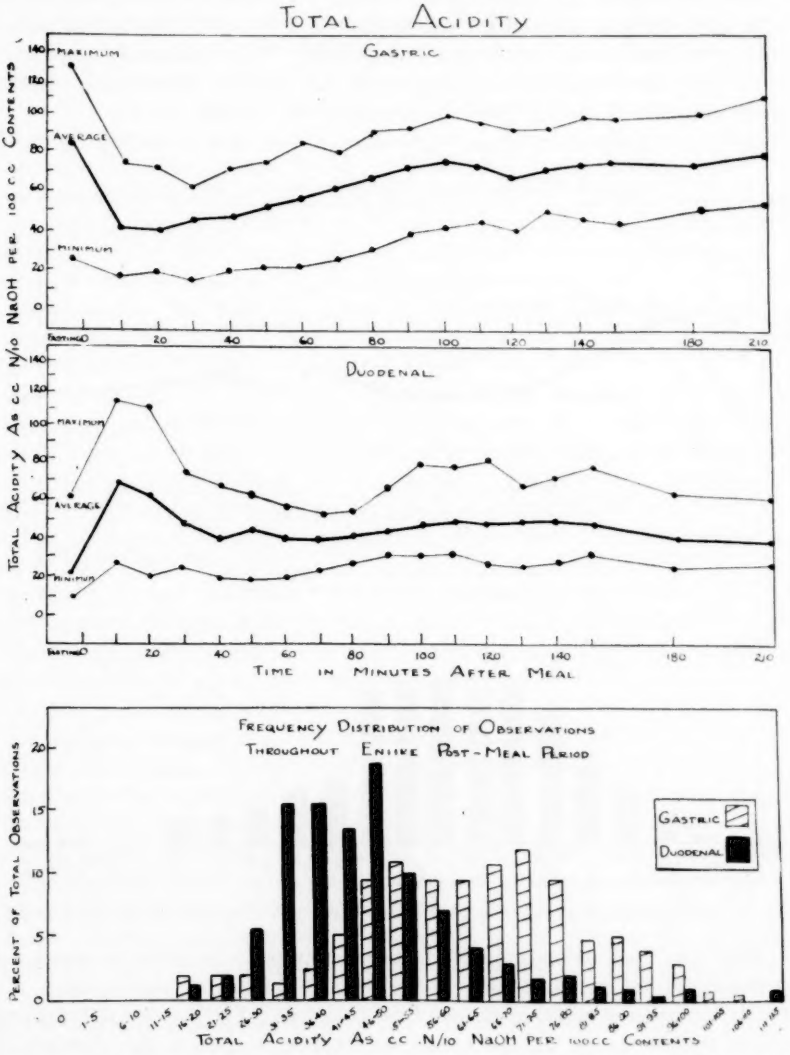

Fig. 3. Total acidity of samples collected simultaneously from just above and just below the pylorus.

and is greater than that subsequent to carbohydrate-water stimulation. Most of the values, furthermore, cluster about a higher range than do those with either of the other stimuli. This may represent the effect of the buffer action of the

soaps contained in the duodenal contents as a result of the interaction between the fatty acids and the alkaline salts of the secretions.

The curve obtained by plotting the average values of duodenal total acidity simulates slightly, if at all, that seen in the stomach and in the latter part of the post-meal period diverges from it completely. This relationship emphasizes further the lack of constant or close correlation between the acidity simultaneously determined in the pyloric and the duodenal contents.

A peculiar phenomenon whose explanation is not clear is the initial rise in

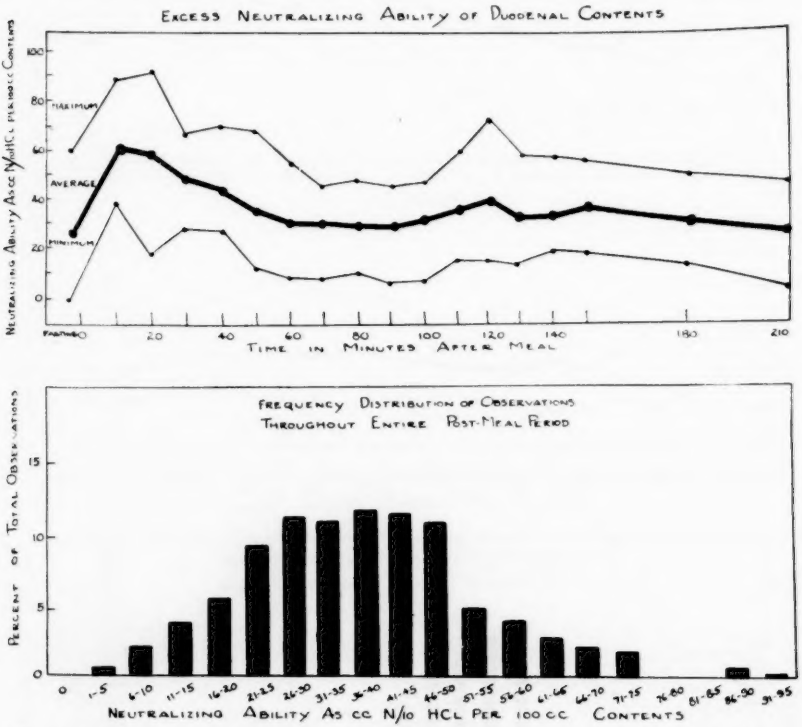
Excess Neutralizing Ability of Duodenal Contents
Neutralizing Ability As cc N/10 HCl per 100 cc Contents
Maximum
Average
Minimum
Time in Minutes After Meal
Frequency Distribution of Observations
Throughout Entire Post-Meal Period
Percent of Total Observations
Neutralizing Ability As cc N/10 HCl Per 100 cc Contents

Fig. 4. Excess neutralizing ability of the contents of the first part of the duodenum.

total acidity in the duodenum in the first 20 minutes postprandial as compared with the initial fall in total acidity in the stomach during the same period.

Excess neutralizing ability of the duodenal contents. (Fig. 4.) As defined and described previously (2, 3), the excess neutralizing ability of the contents of the duodenal bulb is a measure of the reserve capacity which these contents possess to neutralize, buffer and dilute the chyme received from the stomach above that necessary to offset the free acid content. It was estimated by adding N/100 hydrochloric acid to a 1 cc. sample of the strained, admixed duodenal contents containing Toepfer's reagent until the color end point used for the titration of free acid was reached.

The average excess neutralizing ability of the duodenal contents was a little greater than that after the Ewald and beef extract-histamine meals; the range of the values was as great as with the bread and water stimulus and most of the values clustered about a higher range than those following the other meals. Moreover, none of the specimens failed (colorimetrically) to show some excess neutralizing capacity, whereas 2.7 per cent of the post-Ewald and 13.8 per cent of the post-Liebig's extract-histamine meal samples displayed an absence of this reserve capacity.

A remarkable finding was the abrupt initial rise in the average value of the excess neutralizing ability during the first 20 minutes postcibal. This observation coupled with the initial rise in duodenal total acidity without any marked change in the duodenal pH during the same period suggests the presence of an excess of efficient buffer in the duodenal contents at the time.

Discussion. The inhibitory effect of fat on gastric secretion has been long appreciated and amply verified so that the observations made in this study merely lend added confirmation of that action.

One of us (11) previously provided evidence that fat (olive oil or beef fat) modifies gastro-intestinal activity in such a manner as to render the duodenal contents neutral or only slightly acid as determined by the pH of the contents removed from a point about 6 to 8 inches beyond the pylorus. Other investigators have likewise demonstrated a similar decrease in hydrogen ion concentration in approximately the same portions of the duodenum following the feeding of fat in dogs (7) and in man (8). An enhanced neutralizing capacity of the duodenal contents in the same area has also been shown after a fat meal (7). It is of great interest to us, therefore, that this study demonstrates a similar diminution of acidity and an increase in neutralizing ability of the contents of the first part of the duodenum—an area of even greater clinical interest from the standpoint of peptic ulcer.

It has been assumed that the inhibitory influence of fat on gastric secretion is probably mainly responsible for its effect on duodenal pH (7, 11). The lack of any close correlation between the gastric and duodenal pH or total acidity as well as the apparent independence of the duodenal pH and the titratable gastric acidity has been emphasized in the description of our results. Such findings suggest that the degree of gastric acidity is not quite so dominant a factor in determining the duodenal reaction as it has been considered. The effective acidity (hydrogen ion concentration) of the contents of the duodenal bulb following a cream meal is probably a resultant of the interaction of several factors chief among which are 1, the amount of acid entering from the stomach; 2, the degree of pancreatic response (8), especially of bicarbonate; 3, the buffer effect of the soaps formed in the duodenum; 4, the amount of weak, fatty acids contained; 5, and the diluent effect of the liquid meal.

Cream exerted a greater depressant effect on duodenal acidity both as regards the average values and the distribution curves than did meat (12), carbohydrate (2) or Liebig's extract and histamine (4). Moreover, it appears that cream has even a greater effect on duodenal than on gastric acidity. These findings coupled with the lack of sharp relationship between the gastric and

duodenal acidities lend support to the conclusion that the acidity of the duodenal contents in the normal dog is largely determined by the type of food undergoing digestion and is related in part only to the acidity of the gastric contents.

The effect of some of the commonly used antacids on the acidity of the contents of the duodenal bulb in duodenal ulcer patients has been studied by us and will be subsequently reported. Their action for the most part proved to be short and often negligible. The much more efficient action of cream in reducing duodenal acidity over a prolonged period in dogs suggests the preferential use of this foodstuff when a duodenal antacid effect is desired. It is quite true that the amount of cream used in these experiments is much greater than that which would be employed in therapy in patients. The small doses which would be required might prove to be ineffective, yet there is evidence that small doses of fat (olive or cod-liver oil) depress gastric pouch secretion effectively and over long periods (6) so that one might expect a similar effective action in the duodenum from small doses of cream.

SUMMARY AND CONCLUSIONS

1. Fat in the form of table cream (20 per cent fat) effectively diminishes the acidity and enhances the excess neutralizing ability of the contents of the first part of the duodenum over fairly prolonged periods of time in the normal dog.

2. The decrease in duodenal acidity following cream is only partly due to the inhibitory effect of the fat on gastric secretion.

3. There is no parallel relationship between the effective acidity (hydrogen ion concentration) of the duodenal contents and any of the customary measures of gastric acidity in the normal dog.

4. The acidity of the contents of the duodenal bulb in the normal dog is largely determined by the type of food undergoing digestion and is related in part only to the acidity of the gastric contents.

5. Cream appears to be a much more effective duodenal "antacid" than some of the commonly employed antacid medications.

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PRECOCIOUS GONADAL DEVELOPMENT OCCURRING IN IMMATURE RATS FOLLOWING A SHORT-TIME TREATMENT WITH ANTIGONADOTROPIC SERUM[1]

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The administration of gonadotropic extracts to immature rats results in an increase in weight of the gonads that is only temporary in nature and is not accompanied by normal cyclic changes in the vaginal smears. It is conceivable, however, that a premature stimulation of the ovaries with continuous normal cyclic fluctuations in the vagina might follow gonadotropic hypersecretion of the animal's hypophysis. In preliminary reports (1) we have shown that an increase in the number of basophiles in the pituitary gland of the rat occurs during antigonadotropic treatment. After cessation of treatment there is a hypersecretion of the gonadotropic hormone accompanied by a decrease in the number of basophiles (1, 2). Severinghaus and Thompson (3, 4) have also reported basophilism in the pituitary gland of animals repeatedly injected with either sheep pituitary extracts or antigonadotropic sera. Inasmuch as an increased secretion of the pituitary gonadotropic complex follows antihormone treatment it became of interest to ascertain whether premature development and function of the gonads would follow in immature female rats after administration of antigonadotropic serum. This report extends the preliminary observations already made (1, 2) on the effect of antigonadotropic substances upon the physiology and histology of the pituitary gland.

PROCEDURE AND RESULTS. The source of the antihormone used in these experiments was an aspecific gonadotropic antiserum obtained from the blood of rabbits repeatedly injected with an aqueous supercentrifuged extract of sheep pituitary glands. The blood was removed by cardiac puncture and the serum prepared by centrifugation. The inhibitory action of the serum was determined by injecting the serum together with each of the following gonadotropic preparations: extracts of whole sheep pituitary glands, pregnancy urine and pregnant-mare serum and aqueous suspensions of dried human and rat hypophyses. In addition to preventing the gonad stimulating action of these extracts the antiserum also inhibited the endogenous gonadotropic secretion of rat hypophyses (5).

Immature female rats were injected subcutaneously with 0.5 cc. of the antigonadotropic rabbit serum per day—or a total dose of 5 cc.—from the 10th to the 19th day of life, inclusive, to determine the effect of gonadotropic antisera upon the pituitary gonadotropic secretion. Nine of these animals were killed on the 20th day. The average ovarian weight of the injected animals was

[1] Aided in part by a grant from the Wisconsin Alumni Research Foundation and by assistance given by the personnel of the W.P.A. Official Project no. 65-1-53-2349.

5.3 mgm. as compared to 8.9 for 8 untreated littermate control animals killed at the same time. Sections of the gonads of the animals given antihormone did not show follicles with antra which are found in the ovaries of control animals of the same age. The condition of the ovary in these animals indicates that the gonad stimulating hormone of the pituitary gland was prevented from producing its characteristic effect.

The pituitary glands of the antihormone treated and control animals were removed at autopsy and fixed in Bouin's fluid. They were sectioned at 6 microns and stained with Rasmussen's modification of Mallory's trichrome stain (6).

TABLE 1

Increase in ovarian weight of immature rats following cessation of antigonadotropic treatment

TREATMENT	NO. OF RATS	DAY KILLED	OVARIAN WEIGHT	UTERINE WEIGHT
		age	*mgm.*	*mgm.*
Experimental*	9	20	5.3	12.4
Control	8	20	8.9	19
Experimental	7	22	7.8	17
Control	7	22	10.6	19
Experimental	7	24	15	39
Control	5	24	11	20
Experimental	7	26	21	87
Control	6	26	12	31
Experimental	7	28	27	119
Control	4	28	13	26.5
Experimental	8	31	48	
Control	8	31	15	
Experimental	7	34	54	148
Control	5	34	16	29

* Injected with sheep antigonadotropic rabbit serum from the 10th to the 19th day of life, inclusive.

The pituitary glands of the rats treated with the antigonadotropic serum exhibited a picture of extreme basophilism when compared with those of littermate controls. The degree of basophilism found in the injected rats was the same as that found in castrated rats. The results of a quantitative study of the changes in the three cell types of the pituitary gland have been presented in an earlier paper (1) where it was reported that after a 10 day period of antihormone injections 31.9 per cent of all the cells were basophiles, in contrast to 5 to 10 per cent found in the littermate controls. A pituitary gland showing such a degree of basophilism could be expected to exhibit an increase in gonadotropic secretion (7). Hence upon withdrawal of the antiserum further neutralization of the endogenous

gonadotropic hormone by the antiserum would be prevented and the gonad stimulating hormone secreted by the basophiles could act on the gonads. With this thought in mind, rats treated with 0.5 cc. of antigonadotropic serum per day from the 10th to the 19th day of life, inclusive, and uninjected littermate controls were killed 1, 3, 5, 7, 9, 12 and 15 days after cessation of the 10 day treatment with antiserum. The data in table 1 show that the ovaries and the uteri of the animals killed at successive intervals after antihormone treatment exhibited a progressive increase in weight from a subnormal value found immediately after the injections were discontinued, to one much greater than that found in control animals. Accompanying the increase in weight and activity of the ovaries of the antihormone treated animals there was a progressive decrease in basophilic cells until the 15th day after the injections were discontinued when the normal picture again prevailed. The reduction in number of basophiles may be the result of the ovarian secretions produced after the antihormone treatment was stopped. This theory is supported by results obtained in an experiment in which all the animals in three litters of six female rats each were treated with 0.5 cc. of antigonadotropic serum per day from the 10th to the 19th day of life inclusive. One third of these animals in each litter was killed on the 20th day of life; another third was castrated on the 20th day and was killed with the remaining rats on the 34th day of life, or 15 days after cessation of injections. The pituitary glands of the antihormone treated animals which were castrated immediately following the last injection exhibited approximately the same degree of basophilism noted at the time that the injections were stopped. A normal picture was found in the pituitary glands of littermate females that were not castrated after cessation of antihormone treatment, but were killed on the 34th day of life. The average ovarian weight of these animals had increased to 63 mgm. as compared to the control ovarian weight of 16 mgm. The enlargement of the ovaries was undoubtedly accompanied by an increase in the amount of gonadal hormones which might be the cause of the concomitant decrease in the number of basophiles.

Forty-two additional female rats were treated from the 10th to the 19th day of life, inclusive, with 0.5 cc. of antigonadotropic serum per day to obtain further evidence concerning the induced physiological activity of the pituitary gland of the antihormone treated animal as determined by the effects on the gonads and vaginal smears. The time of vaginal opening after cessation of antigonadotropic injections on the 20th day was noted for all the experimental females. Twenty-four of these animals were killed 5 days after opening of the vagina to determine the extent of ovarian hypertrophy present at that time. Similar observations were made of littermate control rats of the same age. The data in table 2 show that the average age of rupture of the vaginal membrane of the 24 antihormone treated animals was 28.5 days and the ovarian weight 5 days later was 42 mgm. Eighteen littermate control rats killed at the same time had an average ovarian weight of 16.1 mgm. The ovaries of the experimental rats showed large cystic follicles, corpora lutea and blood points, whereas those of the control animals were juvenile in appearance. Since the control animals were

killed prior to the time when opening of their vaginas would have normally occurred, the time of opening in the experimental rats treated with antihormone could not be compared with that of littermates. It was necessary, therefore, to make the comparison with non-littermate rats injected with normal rabbit serum. As the average age of vaginal rupture of these animals was 48 days (table 2) it is apparent that the sexual development of the antihormone pretreated animals was accelerated 19 days before its normal occurrence.

The 18 remaining rats that were treated from the 10th to the 19th day of life, inclusive, with antihormone were not autopsied until at least 70 days of age. Daily vaginal smears were made from the time that the vagina opened until

TABLE 2

Response of the immature rat to pretreatment with antigonadotropic sera

TREATMENT	NO. OF RATS	TOTAL DOSE	OVARIAN WEIGHT	UTERINE WEIGHT	AGE AT O. V.†
		cc.	*mgm.*	*mgm.*	
AntiSAP* 10th to the 19th day of life, inclusive, and killed 5 days after O.V.†	24	5	42		28.5
Littermate controls	18		16.1		Not open
AntiSAP 1st to the 10th day of life, inclusive, and killed 5 days after O.V.	13	1	17	124‡	21.5
Littermate controls	12		15.5	25.6‡	Not open
Normal rabbit serum 10th to the 19th day of life, inclusive, and killed at 41 days of age	11	5	17		Not open
Littermate controls	7		17		Not open
Normal rabbit serum 10th to the 19th day of life, inclusive	14	5			48.2
Littermate controls	11				47

* Sheep antigonadotropic rabbit serum.

† Opening of the vagina.

‡ Three rats.

autopsy. Littermate control rats were likewise observed and both the experimental and control animals were killed at the same age after recording at least three successive estrous periods for the control animals. The vaginal smears showed that the experimental animals experienced at least one or two successive estrous periods prior to the time of vaginal opening in the control females. The experimental and control ovarian weights obtained at autopsy were essentially the same, indicating that the hypersecretion of the pituitary gland found soon after stopping the antihormone treatment was only transitory.

In view of these results it became of interest to determine whether the premature opening of the vagina with the accompanying ovarian development and cyclic variations in the vaginal smear could be made to occur earlier by beginning the antigonadotropic treatment of the rats on the first day of life and continuing

it for ten days. With this idea in mind, 35 female rats were injected subcutaneously from the 1st to the 10th day of life, inclusive, with 0.1 cc. per day or a total dose of 1 cc. of antiserum. The time of vaginal opening after cessation of antihormone injection was determined and thirteen animals of this group were killed five days after their vaginas had opened. The results presented in table 2 show that pretreatment with antigonadotropic serum from the 1st to the 10th day of life, inclusive, with a total dose of 1 cc. of serum was followed by a precocious opening of the vagina and development of the uterus, but not by any significant increase in ovarian weight over that seen in littermate controls.

Daily vaginal smears were made from the first day of rupture of the vaginal membrane of littermate control rats and of the remaining rats which had been treated with antigonadotropic serum from the 1st to the 10th day of life, inclusive. Vaginal smears were taken from both groups until at least three vaginal estrous periods had been recorded for the control animals. The antihormone treated rats experienced vaginal estrous cycles beginning on the 22nd day to the 25th day of life and of these, thirteen rats had cycles comparable to those occurring in littermate control rats between the 45th and 50th day of age.

The serum used in the above experiments, as already noted, was obtained from rabbits repeatedly injected with sheep pituitary extracts. In order to eliminate the possibility that a non-specific substance in the serum might be the cause of the precocious sexual development, 0.5 cc. per day of normal rabbit serum was administered to 25 female rats from the 10th to the 19th day of life, inclusive. Eleven of these animals were killed on the 41st day of life to determine whether any gonadal stimulation followed cessation of treatment. The data in table 2 show that there is no significant difference between the average ovarian weight of the animals treated with normal rabbit serum and that of the untreated normal littermate females killed at the same age. The time of rupture of the vaginal membrane of the remaining 14 females that received normal rabbit serum was compared with that of untreated littermate control rats. The vaginas of the injected rats opened at an average of 48.2 days, while those of the untreated females opened at 47 days of age.

Discussion. The data presented in this paper demonstrate that precocious gonadal activity, as evidenced by ovarian hypertrophy and premature establishment of the estrous cycles, follows treatment with antigonadotropic rabbit serum. The effect is not the result of the presence of gonadotropic substances in the antisera of the rabbits, for the ovaries of the animals injected with the antisera became atrophic while the serum was being administered. Likewise, the increase in size and function of the ovaries cannot be explained on the basis of the foreign protein of the serum since no acceleration or inhibition of gonadal development was obtained with normal rabbit serum. The explanation of the mechanism whereby gonadal stimulation follows antihormone administration is based upon the theory already suggested by us in a previous report (1). Briefly it is as follows: As a consequence of the antihormone injections the gonadotropic secretion of the animal is rendered ineffective, thereby causing an atrophy of the gonads. Continued administration of the antihormone is followed by further

atrophy of the gonads, which eventually results in a condition similar to that seen after hypophysectomy. As a result of the atrophied gonads there is an increase in the number of basophiles and the amount of gonadotropic secretion in the pituitary gland. The increased secretion of the basophilic pituitary, however, is prevented from reaching the gonads during the period of antihormone injection, but when the antihormone is withdrawn the hypersecretion of the gonadotropic hormone is no longer neutralized and the gonads are accordingly stimulated to a degree far beyond that of animals of the same age. Accompanying the increase in size and function of the ovary there is a gradual change in the castrate-like pituitary from a state of extreme basophilism to one of normal cell composition. Other experiments, however, showed that when the ovaries are removed at the end of antihormone treatment the basophilism found immediately after cessation of injections was maintained. In view of these results the gradual decrease in basophilism of the pituitary gland may be explained on the basis that the basophiles revert to chromophobes (7) and the ovarian hormones produced by the stimulated ovary prevent the differentiation of more than the normal number of chromophobes into basophiles.

SUMMARY

Hypersecretion of the gonadotropic hormone by the pituitary gland of immature female rats followed treatment with antigonadotropic serum. The hypersecretion resulted in a precocious development of the ovaries and premature occurrence of the cyclic variations in the vaginal smears of the treated rats after discontinuing the injections of the antigonadotropic serum. Extreme basophilism was found in the pituitary gland during treatment with the antigonadotropic sera but when the injections were discontinued the number of basophiles decreased to normal. This condition was accompanied by a marked increase in size and function of the ovaries. In control experiments the injection of rats with serum from rabbits not treated with gonadotropic extracts was without stimulatory or inhibitory effects upon the gonads.

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PLASMA PROTEIN REPLACEMENT AFTER HEMORRHAGE IN DOGS WITH AND WITHOUT SHOCK

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Clinical and experimental studies of shock have emphasized the importance of the plasma proteins in maintaining an adequate plasma volume. In shock associated with trauma, burns and peritonitis, the plasma proteins are greatly diminished. Because the fluid in the traumatized, burned, or infected areas has been found to be rich in protein, the low plasma protein has been attributed primarily to the leakage of plasma protein from the blood stream into the tissues. It is obvious, however, that the quantity of plasma protein in the blood stream depends on the sum of two factors—i.e., the amount of protein added to the blood stream and the amount of protein lost from the blood stream. It seemed possible that failure of plasma protein production was an important factor in the marked decrease in the level of the plasma protein which is observed in shock. The purpose of this paper is to determine whether shock significantly affects protein regeneration.

Acute hemorrhage served as a means both for stimulating plasma protein production and for producing shock. The amount of protein that the dogs produced in the 12 hours immediately after venesection was determined *a*, when the hemorrhage was not severe enough to produce shock; and *b*, when the hemorrhage was severe enough to produce shock. In the latter experiments circulatory insufficiency was maintained by the removal of more blood whenever the circulation improved. The total plasma protein was measured before the bleeding and 12 hours after the first bleeding. The amount of protein produced was calculated by subtracting the total circulating plasma protein before hemorrhage from the sum of the plasma protein removed plus the circulating plasma protein 12 hours after the initial hemorrhage.

METHOD. The experiments were performed on normal unanesthetized adult mongrel dogs weighing 14 to 21 kgm. They ate a diet of cooked horse meat for at least 2 weeks before any observations were made, but were allowed no food for 36 hours before an experiment. Water was not restricted except for about one hour prior to and during the experiment.

The plasma volume was measured by the dye method (1, 2). Heparin was usually used as the anticoagulant, although a 1.6 per cent solution of potassium oxalate was used in 3 experiments. All samples of blood were taken without stasis. The total protein concentration in grams per cent was calculated from the protein nitrogen as determined by a standard micro-Kjeldahl technique (3). All nitrogen determinations were done in duplicate. The total circulating

plasma protein in grams was calculated by multiplying the protein concentration in grams per cent by the $\frac{plasma\ volume}{100}$.

The mean arterial pressure was measured in millimeters of mercury by inserting a needle into the femoral artery. In the majority of the experiments, the femoral artery was exposed under local anesthesia. In several experiments in which the circulation remained adequate, arterial punctures were performed without exposing the artery. The initial bleeding was made by puncture of the femoral artery on the unexposed side. Subsequent bleedings were made via the jugular or femoral veins. The blood was pooled in a flask containing a solution of 1.6 per cent potassium oxalate. Knowing the volume of fluid, the hematocrit reading and the protein concentration of the plasma-oxalate mixture, the amount of plasma protein removed was easily calculated. A correction was made for the plasma protein removed in sampling.

TABLE 1

Plasma protein replacement after a single large bleeding

DOG	WEIGHT	INITIAL BLEEDING	BASAL				12 HOURS AFTER HEMORRHAGE				SUMMARY		
			Plasma volume	Plasma protein	Total circulating protein	Hematocrit reading	Plasma volume	Plasma protein	Total circulating protein	Hematocrit reading	Protein removed	Protein replaced	Percentage of total circulating protein replaced
	kgm.	*cc.*	*cc.*	*grams per cent*	*grams*		*cc.*	*grams per cent*	*grams*		*grams*	*grams*	
1	18.6	550	890	6.6	59	48	920	4.7	43	31	22	6	10
2	17	455	735	5.7	42	48	760	4.6	35	30	17	10	24
3	18.3	500	1120	5.3	59	39	1100	5.0	55	24	19	15	25
4	18	640	810	6.4	52	47	920	4.9	45	34	22	15	29
5	20.8	490	1200	5.7	68	41	1290	3.9	50	22	27	9	13

Shock was produced by the rapid removal of blood from the femoral artery. It was maintained by further bleeding whenever the animal showed signs of improvement. The details of the method and the criteria used for determining the presence of shock are given below.

RESULTS. *Plasma protein production after hemorrhage without shock.* Plasma protein production was studied in 11 dogs in which the bleeding was not sufficient to produce any marked change in the dog's appearance or behavior. After each bleeding (with the exception of dog 6), an equal amount of normal saline was given intravenously. Some of the dogs had little or no fall in mean arterial pressure. In others there was a decrease of 30 to 40 mm. Hg, but the animals remained active, showed no disturbance of gait, and maintained good muscle tone. In 5 dogs a single large bleeding was done at the beginning of the experiment. A 30 cc. sample of blood for plasma volume determination was removed one hour later. The data are summarized in table 1. These dogs produced from 6 to 15 grams of plasma protein in 12 hours. This amounted to 10 to 29 per cent of their

total circulating plasma protein. In 6 dogs the large initial hemorrhage was followed up by repeated small bleedings throughout the day. In this way much larger amounts of protein were removed without noticeably impairing the circulation. The data are given in table 2. In the 12-hour period these dogs produced from 5 to 15 grams of plasma protein, or from 10 to 30 per cent of their total circulating plasma protein. The amount of protein produced in the 12-hour period could not be correlated with the amount of plasma protein removed.

In 4 of the dogs who had a single large bleeding (dogs 1, 2, 3, 4), the amount of protein produced in the second 12 hours after hemorrhage was also measured.

TABLE 2

Plasma protein replacement after hemorrhage with an adequate circulation and with shock

DOG	EXPERIMENT	DATE	BASAL				12 HOURS AFTER HEMORRHAGE				SUMMARY			PHYSIOLOGICAL SALINE	AVERAGE MEAN BLOOD PRESSURE
			Plasma volume	Plasma protein	Total circulating protein	Hematocrit reading	Plasma volume	Plasma protein	Total circulating protein	Hematocrit reading	Protein removed	Protein replaced	Percentage of total circulating protein replaced		
			cc.	*gms. per cent*	*gms.*		*cc.*	*gms. per cent*	*gms.*		*gms.*	*gms.*		*cc.*	*mm. Hg*
6	I*	6-12-41	960	6.3	60	50	835	4.8	40	39	35	15	25	120	70
	II*	7- 3-41	1025	6.5	67	49	780	4.7	37	35	31	1	1	0	45
7	I	5-20-41	690	6.3	44	55	710	4.9	35	36	22	13	30	700	105
	II	6-26-41	730	6.6	48	53	665	3.5	23	28	26	1	2	700	45
8	I	7- 1-41	925	6.1	56	47	1090	4.2	46	25	25	15	27	1100	105
	II	5-28-41	870	6.6	57	53	670	4.6	31	31	29	3	5	0	65
9	I	7-18-41	950	5.6	53	43	930	4.3	40	22	24	11	21	1140	95
	II	6-19-41	970	5.9	57	48	730	4.1	30	22	32	5	9	250	55
10	I	9- 4-41	745	6.5	48	50	845	4.3	36	32	17	5	10	550	80
	II	7-15-41	690	5.8	40	54	560	3.3	18	28	23	1	3	1160	45
11	I	6-12-41	870	5.9	51	45	980	4.0	39	23	23	11	22	1000	85
	II	7-22-41	810	5.9	48	54	640	4.2	27	29	31	10	21	400	45

* I = hemorrhage without shock.
* II = hemorrhage with shock.

In the first 12 hours these dogs made 6, 10, 15 and 15 grams. In the second 12 hours they made 9, 8, 10 and 11 grams respectively. In the same 4 dogs measurements were also made at approximately one hour after the initial hemorrhage. They had produced 0, 3, 2 and 1 gram respectively. These figures are too small to be significant in view of the errors in the method. They do show, however, that no large amount of protein entered the blood stream immediately after hemorrhage.

Protein regeneration after hemorrhage with shock. Shock from hemorrhage was produced in 6 unanesthetized dogs. The ability of 3 of these dogs to regenerate protein after hemorrhage without shock had been tested from 3 to 5 weeks pre-

viously. In 3 dogs the ability to produce protein after hemorrhage without shock was tested from 3 to 7 weeks after the shock experiment.

Following the initial hemorrhage, the arterial pressure usually dropped to between 20 and 30 mm. Hg. The animals were restless; they voided and defecated involuntarily. In a short time the arterial pressure began to rise and more blood had to be removed. After several hours the arterial pressures became stabilized at a low level which varied from dog to dog. The average arterial pressure was obtained by charting the mean arterial pressure for the 12-hour period. It ranged from 45 to 65 mm. Hg. The dogs were very weak, apathetic, and at times stuporous. During much of the time they were too weak to raise their heads. Chemically the circulatory insufficiency was demonstrated by a rising non-protein nitrogen and a decrease in the CO_2 combining power. In the control experiments, the non-protein nitrogen remained normal and the CO_2 combining power showed little change.

As other investigators have noted, there was no absolute correlation between the mean arterial pressure and the general condition of the dog. Some dogs with a mean pressure of 45 appeared stronger and more alert than others did at a mean pressure of 65. In general, an attempt was made to keep the mean arterial pressure below 50. The best criterion of the condition of the circulation seemed to be the response to the removal of a small amount of blood. If the removal of 20 cc. of blood produced a precipitous fall in arterial pressure, the animal was in a critical state. If it produced no change in arterial pressure, the animal could usually stand further bleeding.

After the first 3 hours little or no blood had to be removed. The animals had to be watched closely because a sudden marked fall in arterial pressure, not produced by further bleeding, frequently occurred, and unless saline was given, they died from cerebral anoxemia. Some of the dogs received as much saline in the course of 12 hours as they did in the hemorrhage experiments without shock. The saline would not restore the circulation to normal after it had been inadequate for several hours, but it would produce sufficient improvement to prevent the death of the animal. All the dogs recovered.

In 5 of the 6 experiments, the dogs in shock produced very little protein in the 12-hour period. The sixth dog produced as much protein as he had after hemorrhage without shock. The average mean arterial pressure in this dog was 45 mm. Hg. It was noted in the protocol, however, that in spite of the low arterial pressure, this dog had been more alert and active than the other dogs. The data are summarized in table 2.

COMMENT. The determination of the plasma volume seemed as satisfactory in the dogs with an inadequate circulation as in normal dogs. Fifteen minutes were always allowed for complete mixing of the dye. In general, the slope of dye disappearance curve was approximately the same before and after the production of shock. The values obtained for the plasma volume and for the total circulating protein seemed reasonable. One hour after hemorrhage the plasma volume was decreased and the total circulating protein was approximately equal to the prehemorrhage total circulating protein minus the amount of protein removed.

Twelve hours after hemorrhage, protein replacement was well under way and, with one exception, the plasma volume had increased to or beyond its prehemorrhage level. Twenty-four hours after hemorrhage a further increase in plasma volume and in total circulating protein had occurred. In the dogs maintained in shock, the plasma volume at the end of 12 hours was always smaller than the original plasma volume. It is possible that the plasma volume of the dogs in shock was smaller during the earlier part of the 12-hour period. During the first 3 to 6 hours, a slight increase in plasma volume produced a marked rise in arterial pressure. After the circulatory insufficiency had been present approximately 6 hours, increasing the plasma volume by saline frequently produced little change in arterial pressure. Freeman (4) has reported similar observations.

Fasting dogs with an adequate circulation vary considerably in their ability to replace plasma protein lost by hemorrhage. There was no correlation between the amount of protein produced and the level of the plasma protein before hemorrhage, or between the amount of protein produced and the amount of protein removed. The diets previous to the control period and the age of the dogs were not known. In the 24-hour period after hemorrhage, protein was apparently added to the blood stream at a fairly constant rate. Three of the 4 dogs tested replaced slightly greater amounts of protein in the first 12 hours than in the second 12 hours after hemorrhage. Too few experiments were done to determine whether this difference was significant.

The rate of mobilization of the protein stores recorded here is in accord with the concept of Madden and Whipple (5), that the greater portion of the protein reserve is not stored as preformed plasma protein. In fact, these experiments demonstrated no measurable reserve of preformed plasma protein which could be added to the blood stream in a few minutes. There was no evidence of a rapid out-pouring of protein in the first hour after hemorrhage. Similar observations have been made after hemorrhage in normal human subjects (6). This is further supported by the observation that 3 of the dogs in shock added only 1 gram of protein to the plasma during a 12-hour period. This question of a small reserve of preformed protein is of considerable importance, because even in the absence of increased capillary permeability, changes in plasma protein concentration would not necessarily reflect changes in the plasma volume if there were a reserve of preformed plasma protein which could be added to the blood stream in a few minutes. Smith, Belt and Whipple (7) have reported a rapid replacement of serum proteins in the first 15 minutes after the replacement of a large portion of the blood by an equal quantity of a red cell Locke's solution mixture. This resulted in an increase in serum protein concentration of about 0.5 gram per cent. As the hematocrit reading showed little rise during this period, and as the rise in fibrin concentration was not proportionally as great as the increase in the serum protein concentration, they concluded that the rapid replacement of serum protein during the first 15 minutes following the exchange indicated some reserve supply of serum proteins probably held in the body cells. In our experiments with dogs and with normal human subjects after bleeding, physiological saline has left the blood stream very rapidly, and the replacement of a quantity of

saline equal to the volume of blood removed has failed to restore the plasma volume to the prehemorrhage level. In the experiments of Smith, Belt and Whipple, the hematocrit readings increased from an average of 53 to an average of 56 in the first 15 minutes. If this increase in hematocrit reading resulted from a decrease in plasma volume, it would account for about one-half of the observed rise in serum protein concentration. In these experiments, one would not have expected the hematocrit reading to parallel the decrease in plasma volume, because a considerable number of traumatized red cells were being destroyed, as shown by the presence of hemolysis in many of the samples and by the subsequent marked fall in the hematocrit reading. In the first 15 minutes, the average increase in serum protein concentration was 23 per cent. At the same time, the fibrin increased an average of 14 per cent. These do not seem significant differences, because an average increase of only 0.01 gram per cent of fibrin would have resulted in a parallel increase in serum protein concentration during the first 15 minutes after the replacement.

The protein replacement in the dogs with shock was significantly less than in the dogs in which the hemorrhage was not accompanied by shock. This is based on the assumption that the protein added to the plasma remained in the blood stream and did not pass into the tissues because of altered capillary permeability. It has been recently demonstrated that the capillaries in shock due to hemorrhage are not abnormally permeable. Price et al. (8) injected a large amount of blue dye, T-1824, into the veins of dogs. This enabled them to observe directly abnormal leakage of dye, and presumably protein, into the tissues. They stated: "Although local staining of tissues was pronounced in areas subjected to trauma of different sorts, no abnormal staining of tissues in general was observed in animals dying of post-hemorrhagic shock."

The data indicate that failure of the circulation was the primary factor which prevented normal plasma protein replacement in the dogs in shock. The low protein replacement in the dogs in shock was not caused by lack of adequate fluid intake, because in 2 dogs as much or more saline was administered during the shock experiment as during the simple hemorrhage experiments. The stimulus for protein replacement should have been as great in the dogs in shock as in the dogs not in shock, because the protein concentration was greatly reduced in both groups of experiments. The loss of red cells was not the determining factor, because the hematocrit levels at the end of the 12-hour period were not very different in the two types of experiments. The data in table 2 show that in dogs 6, 7 and 8 there was no great difference in the amount of protein removed in the simple hemorrhage and in the shock experiments. The speed with which the blood was removed, rather than the total amount removed, determined whether shock developed.

After simple hemorrhage the dog first increases his plasma volume by dilution with protein-poor fluids. This restores the circulation to an adequate level and protein replacement proceeds fairly rapidly. Studies in man have shown a similar chain of events (6). In the dog which is kept in shock by repeated small hemorrhages, the dilution with protein-poor fluid occurs, but the further loss of

plasma by bleeding prevents the circulation from becoming adequate for protein regeneration. After the original hemo-dilution has occurred, it is difficult to maintain the plasma volume at a normal level by the administration of normal saline because of the low colloid osmotic pressure of the blood. Therefore, recovery is very slow unless plasma or some other fluid with similar osmotic properties is administered.

It is believed that these experiments which demonstrate the difficulty in protein replacement with a poor circulation offer an adequate explanation for the clinically observed fact that patients with hemorrhage who maintain a good circulation can lose a tremendous quantity of plasma protein and maintain an adequate plasma volume, while patients in certain types of shock have great difficulty in restoring the volume to normal.

SUMMARY

1. Plasma protein replacement after acute hemorrhage, which was not severe enough to produce shock, has been studied in unanesthetized, fasting dogs. One hour after hemorrhage little protein had been added to the plasma. Twelve hours after hemorrhage from 6 to 15 grams of plasma protein had entered the blood stream. This amounted to 10 to 29 per cent of the total prehemorrhage circulating plasma protein. Plasma protein replacement proceeded at the same or at a slightly lower level in the second 12-hour period after hemorrhage.

2. These experiments showed no evidence of a reserve of preformed plasma protein which can enter the blood stream in the first few minutes after hemorrhage.

3. In 6 unanesthetized, fasting dogs, shock was produced and maintained by hemorrhage. Plasma protein replacement was measured over a 12-hour period. Shock usually greatly retarded plasma protein replacement. Dogs which had replaced 15, 13, 15, 11, 5 and 11 grams of plasma protein in a 12-hour period after hemorrhage without shock replaced only 1, 1, 3, 5, 1 and 10 grams respectively in a 12-hour period when they were in shock.

4. The slow rate of plasma protein production with an inadequate circulation explains why the body has great difficulty in restoring the plasma volume to normal in many cases of shock.

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THE RELATION OF THE PITUITARY, THYROID AND ADRENAL GLANDS TO THE MAINTENANCE OF NORMAL SERUM ALBUMIN AND GLOBULIN LEVELS[1, 2]

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Goldberg (1, 2) has reported that hypophysectomy in dogs produces a fall in serum albumin and a compensating increase in serum globulin concentration. Administration of desiccated thyroid to such dogs caused a return of the globulin toward the normal level but had very little effect on the serum albumin concentration. These findings led Goldberg to suggest that the decreased thyroid activity following hypophysectomy is responsible for the changes in serum protein levels.

Before this work came to our attention we (3) had found similar changes following hypophysectomy in the monkey. Transferring our investigations to the rat, we found that in this species hypophysectomy produces serum protein changes similar to those in the dog and the monkey. In the rat, thyroid medication prevents the increase in globulin level but has little if any effect on the albumin level, confirming Goldberg's findings in the dog. These results indicate that some factor, other than the thyroid gland, is responsible for the post-hypophysectomy albumin changes. For reasons to be discussed below, it appeared probable that the adrenal cortical atrophy which follows pituitary ablation might be related to the marked decrease in serum albumin concentration. Attempts were therefore made to study the rôle of the adrenal cortex and to define more clearly the action of the thyroid with respect to maintenance of the serum protein levels. Since a partial inanition occurs after hypophysectomy (4) and since it is known (5) that in certain species alterations of serum protein levels are produced by severe protein inanition, a study of the effect of reduced food intake on the serum protein levels of the rat was also made.

The results of these experiments, forming the basis of this report, indicate that in the rat the maintenance of the serum globulin level is associated with the degree of thyroid activity while the maintenance of the albumin level appears to depend on adrenocortical function. The inanition which follows hypophysectomy, also a factor in the alteration of the serum protein levels, appears to be of lesser importance than does the decreased activity of the adrenal cortex and of the thyroid gland.

EXPERIMENTAL. Adult rats, in most experiments males, of the Long-Evans

[1] Aided by a grant from the Rockefeller Foundation, administered by Dr. P. E. Smith.

[2] A preliminary report of this work was made before the thirty-fifth annual meeting of the American Society of Biological Chemists at Chicago, April 19, 1941 (J. Biol. Chem. **140:** p. lxxvi, 1941).

strain, were used. The animals were maintained under the usual conditions prevailing in our stock colony. The regular adequate stock diet used for our colony, as well as water, was available at all times except as specified below.

The completeness of pituitary removal was verified by microscopic examination of serial sections of the sella. Thyroidectomized animals were examined under the dissecting microscope for thyroid remnants and if any suspicious tissue was seen serial sections of the thyroid region were made and studied. The one case of incomplete thyroidectomy was discarded from the series. In the adrenalectomized animals a gross search at autopsy revealed no cortical tissue but is is realized that accessory cortical tissue might have been present and not seen.

Whenever treatment was given it was begun immediately after operation and was administered by subcutaneous injection daily until the time of removal of blood, in most cases 18 to 21 days post-operatively. Blood was taken directly from the heart which was exposed under amytal anesthesia. A small portion of each blood sample was heparinized and used for hematocrit determination (6). The remainder of the blood was allowed to clot, the serum separated and used for determination of total protein, albumin and non-protein nitrogen. Semi-micro Kjeldahl technic was used for all nitrogen determinations. Albumin filtrates were prepared by the method of Campbell and Hanna (7). The nitrogen values, after correction for non-protein nitrogen, were converted to protein by use of the usual factor 6.25 and globulin was calculated as the difference between total protein and albumin.

RESULTS. The data obtained from untreated normal and hypophysectomized rats are given in table 1, A, B, D, E. The most striking changes resulting from the pituitary ablation are the decrease in serum albumin and the increase in serum globulin. The net result is a decrease in total protein which is relatively slight but statistically significant and a fall in the ratio of albumin to globulin to almost half the normal value.

Following hypophysectomy the animals lost a considerable proportion (males 24.7 per cent; females 14.4 per cent) of the pre-operative body weight. To simulate this weight loss as closely as possible a group of female rats of similar age and weight was subjected to a reduced food intake so that the weight loss (23.6 per cent of the original) approximated in duration and intensity that suffered by the hypophysectomized animals. The changes in serum albumin, globulin, NPN and A/G ratio (table 1, C) are in the same direction but of much lesser magnitude than after hypophysectomy.

The results obtained from thyroidectomized rats are given in table 1, F. The slight decrease in serum albumin is of doubtful significance ($P = 0.05$).[3] The globulin fraction, however, increased to an even greater extent (38.6 per cent over the normal level) than after hypophysectomy (29.1 per cent over the normal

[3] The value designated as "P" represents the probability that the difference between two means is due to random sampling (21). A probability of 0.05 (five chances in 100 that random sampling is responsible for the difference) is frequently selected as a criterion of significance.

TABLE 1

Effect of various treatments on serum protein levels in the rat

TREATMENT	NO.	AGE AT BLEEDING	DURING EXP'T.		CELL VOLUME	NON-PROTEIN NITROGEN	TOTAL PROTEIN	ALBUMIN	GLOBULIN	ALBUMIN / GLOBULIN
			Time	Wt. chg.						
Females										
		days	*days*	*per cent*	*per cent*	*mgm. per cent*	*per cent*	*per cent*	*per cent*	*ratio†*
A. Normal	11	88–137			38.7 ± 0.6	34.1 ± 1.1	5.71 ± 0.08	3.70 ± 0.04	2.01 ± 0.09	1.88 ± 0.09
B. Hypsect., untreated	13	94–136	13–43	−14.4 ± 2.0	32.0 ± 1.4	44.3 ± 2.0	5.30 ± 0.12	2.63 ± 0.06	2.67 ± 0.14	1.02 ± 0.06
Per cent change from normal					−17.3	+29.9	−7.2	−28.9	+32.8	−45.8
P* (vs. normal)					<0.01	<0.01	0.02	<0.01	<0.01	<0.01
C. Intact, partially starved	11	109–225	13–41	−23.6 ± 1.0	37.3 ± 1.0	35.5 ± 1.6	5.70 ± 0.15	3.35 ± 0.12	2.35 ± 0.15	1.49 ± 0.12
Per cent change from normal					−3.6	+4.1	−0.2	−9.5	+16.9	−20.7
P* (vs. normal)					0.30	0.40	0.90	0.02	0.06	0.02
Males										
D. Normal	31	100–164	19–21	+5.9 ± 2.0	43.2 ± 0.6	36.2 ± 1.2	5.88 ± 0.08	3.69 ± 0.06	2.20 ± 0.07	1.73 ± 0.06
E. Hypsect., untreated	36	112–162	18–21	−24.7 ± 0.9	41.3 ± 0.9	44.9 ± 0.9	5.60 ± 0.08	2.76 ± 0.06	2.84 ± 0.08	1.00 ± 0.04
Per cent change from normal					−4.4	+24.1	−4.8	−25.2	+29.1	−42.2
P* (vs. normal)					0.09	<0.01	0.02	<0.01	<0.01	<0.01
F. Thyrect., untreated	11	103–164	20–44	+10.6 ± 5.7	40.9 ± 1.4	38.2 ± 1.0	6.52 ± 0.11	3.47 ± 0.09	3.05 ± 0.15	1.17 ± 0.08
Per cent change from normal					−5.3	+5.5	+10.9	−6.0	+38.6	−32.4
P* (vs. normal)					0.10	0.40	<0.01	0.05	<0.01	<0.01
G. Hypsect., thyroxin treated 10–20 μg. daily	16	124–140	20–21	−29.9 ± 1.3	46.1 ± 0.9	42.5 ± 0.7	5.14 ± 0.08	2.93 ± 0.06	2.21 ± 0.07	1.35 ± 0.06
Per cent change from normal					+6.7	+16.3	−12.6	−20.6	0.00	−22.0
P* (vs. untreat. hypsect.)					<0.01	0.04	<0.01	0.06	<0.01	<0.01
H. Adrex., untreated	6	132–161	19–21	−18.1 ± 0.7	41.0 ± 1.5	71.3 ± 4.6	5.70 ± 0.14	3.09 ± 0.15	2.61 ± 0.06	1.19 ± 0.07
Per cent change from normal					−5.1	+97.0	−3.1	−16.3	+18.6	−31.2
P* (vs. normal)					0.15	<0.01	0.40	<0.01	0.02	<0.01
I. Hypsect., DCA‡ treated 3–5 mgm. daily	12	122–143	18–21	−19.8 ± 1.3	40.6 ± 0.5	37.7 ± 1.0	5.58 ± 0.13	3.02 ± 0.07	2.56 ± 0.13	1.22 ± 0.08
Per cent change from normal					−6.0	+4.2	−5.1	−18.2	+16.4	−29.5
P* (vs. untreated hypsect.)					0.60	<0.01	0.90	0.02	0.08	0.01
P* (vs. normal)					0.01	0.40	0.04	<0.01	0.01	<0.01
Per cent change from normal (corr.)§					0.00	+16.0	+5.5	−9.5	+29.1	−29.5
J. Hypsect., C.E.‡ treated 1–4 cc. daily	17	125–157	18–21	−26.0 ± 0.9	42.4 ± 0.9	45.6 ± 0.9	6.11 ± 0.13	3.33 ± 0.07	2.78 ± 0.13	1.26 ± 0.08
Per cent change from normal					−1.9	+26.0	+3.9	−9.8	+26.4	−27.2
P* (vs. untreated hypsect.)					0.45	0.60	<0.01	<0.01	0.70	<0.01
P* (vs. normal)					0.40	<0.01	0.10	<0.01	<0.01	<0.01
K. Intact Stilbestrol 1 mgm. daily	12	121–157	21	−25.9 ± 1.9	33.6 ± 1.0	39.5 ± 1.1	6.22 ± 0.10	4.09 ± 0.09	2.13 ± 0.06	1.94 ± 0.07
Per cent change from normal					−22.2	+9.1	+5.8	+10.8	−3.2	+12.1
P* (vs. normal)					<0.01	0.10	0.02	<0.01	0.50	0.05

The ± values are for the mean deviation of the mean, calculated as $\varepsilon_m = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

* P expresses the probability that the difference between two means is due to random sampling (see footnote 3).

† The values given for A/G ratio are the means of the individual A/G ratios rather than the ratio of the mean albumin to mean globulin.

‡ DCA = desoxycorticosterone acetate. C.E. = adrenal cortical extract.

level). The implications of these results are reinforced by the results obtained from animals treated with 10 to 20 μg. of thyroxin daily from the time of hypophysectomy until bleeding (table 1, G). Although the thyroxin treatment did not prevent the decrease in serum albumin which followed hypophysectomy, the increase in the globulin fraction was entirely prevented.

Because changes in thyroid activity appear to have very little effect on the serum albumin level, a series of experiments was made to investigate the relation of adrenal cortical action to the serum proteins. Although complete adrenalectomy is almost impossible in the adult rat, a small group of animals were adrenalectomized with the hope that the chronic partial adrenal insufficiency might show some effect. It should be emphasized that a considerable proportion of such animals died before the routine three weeks postoperative period had elapsed. It seems justifiable to assume that these suffered the highest degree of cortical insufficiency. Therefore, any serum protein changes found in the surviving rats are probably representative of only the milder forms of cortical insufficiency. It may be noted (table 1, H) that the animals lost considerable weight and yielded low serum sodium (average 298.4 mgm. per cent) and high serum NPN values. Surprisingly, the hematocrit readings showed no significant change from normal. The changes in serum protein levels are similar in direction to those following hypophysectomy but are of considerably lesser magnitude. As compared to values obtained from intact rats, the albumin is significantly decreased ($P = < 0.01$) and the globulin increased ($P = 0.02$).

In order to evaluate more completely the effect of the cortical insufficiency which follows hypophysectomy, two series of hypophysectomized rats were given cortical replacement therapy daily throughout the post-operative period. One group (table 1, I) was given desoxycorticosterone acetate (3.0–5.0 mgm. daily) while the other group (table 1, J) was treated with adrenal cortical extract (1.0 to 4.0 cc. daily).[4] The DCA treatment did not prevent the weight loss which follows hypophysectomy. However, it did partially but significantly ($P = 0.02$) prevent the decrease in serum albumin level which occurs in untreated hypophysectomized animals. The protection against serum globulin increase was not significant ($P = 0.08$). It should be noted that the hematocrit reading of the DCA treated animals is significantly below that of the normal rats ($P = 0.01$). If the assumption is made that this decrease in hematocrit is due to hemodilution and an appropriate correction is made, it may be seen (table 1, I) that the post-hypophysectomy change of albumin concentration is largely prevented (9.5 per cent below normal) by the DCA while the globulin change (to 29.1 per cent above normal) is almost identical with that of untreated hypophysectomized rats.

Treatment with cortical extract did not prevent the post-hypophysectomy

† The values given for A/G ratio are the means of the individual A/G ratios rather than the ratio of the mean albumin to mean globulin.

[4] The authors wish to express their thanks for generous supplies as follows: For adrenal cortical extract to Wilson Laboratories, through Dr. David Kline, and to the Upjohn Company, through Dr. G. F. Cartland. For desoxycorticosterone acetate to Ciba Pharmaceutical Products, through Dr. R. C. Mautner, and to the Schering Corporation, through Dr. Erwin Schwenk. For stilbestrol to E. R. Squibb and Sons, through Dr. J. A. Morrell.

weight loss nor the increase in serum NPN level. However, the decrease in serum albumin was largely prevented ($P = < 0.01$) while the globulin increase was not significantly different ($P = 0.70$) from that of untreated animals, the net result being a total protein value higher than that of either normal or hypophysectomized rats.

Stilbestrol, administered to normal rats as a corticotropic agent (1.0 mgm. daily) produced adrenal enlargement and hyperemia as previously reported by Selye (8) and many others. Despite the rather severe inanition suffered by these animals (table 1, K) the serum albumin level rose considerably above the normal value while the globulin level remained almost constant. The hematocrit value fell considerably.

Discussion. Following hypophysectomy the adult rat suffers a pronounced loss of body weight which is associated with a decreased food intake (4) and an impaired absorption from the gastro-intestinal tract (9, 10). That the resultant inanition is at most only partially responsible for the serum protein changes is shown by the finding that in intact rats inanition of somewhat greater severity than that following hypophysectomy produced less than half as much change in serum protein levels. This is confirmed by the data obtained from normal rats treated with stilbestrol. Such treatment causes a marked loss of body weight which is almost certainly a result of the decreased food consumption for Ingle (11) has shown that forcibly feeding such animals produces a weight gain instead of a loss. Despite the severe inanition suffered by our stilbestrol treated rats, the serum albumin concentration actually increased while that of the globulin remained constant. It may also be noted that hypophysectomized rats treated with adrenal cortical extract suffered as severe a weight loss as did the untreated operated animals. The serum albumin concentration, however, decreased only slightly thus confirming the fact that during short experimental periods the changes in serum protein levels are not necessarily proportional to the degree of inanition.

Our results support Goldberg's suggestion (1, 2) that the thyroid inactivity which follows hypophysectomy is responsible for the increase in the serum globulin levels. However, neither his data nor ours justify the belief that the lack of thyroid activity is responsible for the change in albumin concentration. Administration of thyroxin to hypophysectomized rats has little if any effect on the decrease in albumin level. Nor does removal of the thyroid produce a pronounced albumin decrease as occurs after hypophysectomy. It seems quite definite, therefore, that the thyroid is in some way involved in the maintenance of the serum globulin level but exerts little, if any, control over the serum albumin metabolism.

The adrenal cortex is known to have an important influence on salt and water metabolism and to exercise control over the distribution of body fluids and the maintenance of blood volume (12, 13). It is also known to be intimately concerned with the intermediary metabolism of proteins (14). This knowledge, taken together with the fact that the serum albumin is one of the most important factors in the maintenance of blood osmotic pressure (5, 15) and, therefore, of

blood volume, indicates the possibility of a direct relationship between adrenocortical activity and serum albumin metabolism.

Treatment of hypophysectomized rats with cortical substances yields evidence supporting this hypothesis. Thus, administration of adrenocortical extract largely prevents the fall in serum albumin which occurs in untreated animals. The increase in serum globulin, however, is not prevented. When desoxycorticosterone is used for replacement therapy almost identical results are obtained if the lowered hematocrit is taken into account. Such a correction of values for hemodilution is not illogical in the light of the well known blood diluting effect of desoxycorticosterone.

The treatment of hypophysectomized rats with the cortical substances did not completely maintain the serum albumin concentration at its normal level. It must be remembered that the dosages used, as well as the mode and spacing of the injections, may have been far from optimal. Furthermore, some other of the substances now known to be elaborated by the adrenal cortex may be more effective than the substances used in the present experiments. In this connection it is well to recall the different potencies of these substances with respect to carbohydrate metabolism, salt and water metabolism, etc. (14, 16, 17, and others).

The treatment of normal rats with stilbestrol provides further evidence indicating control of serum albumin metabolism by the adrenal cortex. Stilbestrol appears to be a potent stimulant to the adrenal cortex (8, 11, and many others) and might be expected, therefore, to produce an increase in the serum albumin concentration. Such an increase would be of limited magnitude because it, *per se*, would tend to cause a porportional hemodilution. The results obtained were as anticipated, the albumin level of the treated animals being slightly (10.8 per cent) but significantly ($P = 0.01$) above the normal level. The globulin level did not change appreciably as would be expected if the adrenal cortex controls albumin but not globulin metabolism. The hematocrit value was markedly lower than normal, possibly a consequence of decreased hemopoiesis (18, 19) or of hemodilution induced by the increased concentration of the serum albumin.

The results obtained from the small group of adrenalectomized rats do not entirely fit into the general picture. In this case decreased albumin and increased globulin levels were found, indicating no preferential control. However, as noted in a previous section of this paper, the cortical insufficiency exhibited by the animals which survived the entire experimental period is probably very mild. Furthermore, the animals apparently suffered a considerable loss of appetite as reflected by the loss of body weight. It is not improbable, therefore, that the observed changes are the combined effect of inanition and mild cortical insufficiency. The globulin change is very nearly the same as that suffered by animals subjected only to inanition. The albumin decrease is somewhat greater than that produced by inanition alone and may be interpreted as the combined effect of the inanition plus the mild cortical insufficiency. In this connection may be mentioned the results obtained from two small groups of adrenalec-

tomized rats, one treated with desoxycorticosterone acetate (2.5 mgm. daily) and the other drinking 1 per cent NaCl solution instead of tap water. In each case the animals showed a small weight gain as contrasted to the weight loss suffered by untreated adrenalectomized rats. Other changes shown by the untreated animals were completely prevented with the exception of the decrease in serum albumin level in the case of the group drinking 1 per cent NaCl. The albumin level of these rats was 8.1 per cent below normal ($P = 0.03$). This may be interpreted to mean that the DCA therapy completely replaced the lost adrenocortical function but that increasing the NaCl intake, while preventing the loss of appetite, did not replace the adrenal cortex insofar as maintenance of serum albumin level is concerned.

The concept of maintenance of serum albumin by adrenocortical influence is not inconsistent with the recent findings of Long (14, 20) which indicate that the adrenal cortex controls general protein catabolism. According to Long, adrenocortical activity favors breakdown of body protein to amino acids of which a portion are converted to carbohydrate. It is not unlikely that another portion of these newly formed amino acids may be used to replenish or replace other vital substances of which serum albumin is representative.

The authors wish to express their thanks to Dr. G. K. Smelser for performing the thyroidectomies reported in this paper and to Mrs. Elizabeth Wolfe for valuable technical assistance.

SUMMARY AND CONCLUSIONS

1. Hypophysectomy in rats produces a fall in serum albumin and an increase in serum globulin with a slight resultant decrease in total serum protein. These changes are reproduced to only a slight extent by inanition similar in duration and intensity to that suffered by the hypophysectomized rats.

2. Thyroidectomy simulates hypophysectomy in so far as increase of serum globulin is concerned. There is very little, if any, effect on the serum albumin level. Conversely, treatment of hypophysectomized rats with thyroxin prevents the increase in serum globulin but does not inhibit the decrease of the serum albumin level.

3. Treatment of hypophysectomized rats with adrenal cortical extract or with desoxycorticosterone, in the doses used, to a large extent prevents the decrease in albumin level with little or no effect on globulin level.

4. Stilbestrol, administered to intact rats, causes an increase in serum albumin level, presumably via the adrenal cortex which is markedly stimulated. There is no effect on the globulin level.

5. The above findings lead to the tentative conclusion that in the rat the serum albumin level is maintained under adrenocortical influence while the serum globulin level is associated with activity of the thyroid gland. Following hypophysectomy, a change in both fractions occurs because of the decrease in both adrenocortical and thyroid activity.

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LINEAR RELATIONSHIP BETWEEN THE CIRCULATING RED CELL MASS AND THE VENOUS HEMATOCRIT AS DETERMINED WITH RADIOACTIVE IRON

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When iron tagged with radioactive isotope[1] is administered under such conditions that the absorbed iron is utilized immediately for red cell formation, the concentration of tagged iron in the blood stream may remain quite constant for many months (3). Exchange of the radio iron of the blood stream does not occur with other iron of the body (5) and it seems that the isotope once incorporated into the red blood cell remains there until the cell undergoes disintegration (1, 3). Thus, under conditions under which no destruction of the labeled cells is occurring, it would seem that the total labeled iron in the circulation, once established, remains constant.

It is therefore possible to study the relationship between circulating red cell mass and jugular hematocrit at various hematocrit levels by using the radio iron-labeled-donor cell method described in detail elsewhere (6, 7). This method consists essentially of a transfusion from a donor animal of a few milliliters of blood containing an accurately known amount of iron built into red cells, and then by measuring the radioactivity of subsequent blood samples to determine the dilution that has occurred.

It is to be expected that increased jugular or venous hematocrit indicates an increased mass of red blood cells and hemoglobin in the experimental animal or patient. There are data on one dog (3) to indicate that the circulating red cell mass is quite accurately proportional to the jugular hematocrit. However, this donor cell technique affords a method for determining on an individual animal the red cell mass at a large number of widely different hematocrit levels. Data are therefore here presented on results of 24 pairs of determinations of hematocrit and cell mass on three dogs with hematocrit readings varying from 11 to 57 per cent.

METHODS. The animals used were all adult mongrel dogs which had been vaccinated against distemper. Their care has been described elsewhere (8). Diet consisted of hospital table scraps.

Plasma volume was done by a modification of the Brilliant Vital Red dye procedure (9). Samples were taken from the jugular veins on the same side of the neck as was used for the dye injection, the needle being flushed with blood occasionally. Stasis was avoided. Amounts of dye were injected such that the final dilutions resulted in readings of both standard and unknown in the colori-

[1] We are indebted to the members of the Radiation Laboratory at Berkeley, California, and in particular to Dr. E. O. Lawrence and Dr M. D. Kamen for the radioactive iron used.

meter within 10 per cent of one another, these amounts being determined previously for each dog. A single dyed sample was taken at the end of four minutes after injection of the dye.

Hematocrits were determined on the samples taken above, the blood being taken in 2 ml. of isotonic (1.4 per cent) sodium oxalate in 15 ml. centrifuge tubes and spun at about 2700 rpm. for 35 minutes.

Red cell circulating volumes were determined as follows: An initial sample of 30 ml. of blood was drawn into 5 ml. of isotonic oxalate. Twenty-five milliliters of blood containing citrated radioactive red cells from a donor were then given by vein through the same needle within thirty seconds' time. After 15 minutes during which the animal was permitted to stand in a cage, another 30 ml. of blood were drawn as before. The blood was divided in each case so there were triplicate samples for determination of isotope activity. The aliquots were centrifuged for 35 minutes and the hematocrits of each read and averaged. The red cells were then ashed and the iron separated for electroplating (3) and activity determinations (3, 4). An aliquot representative of the injected blood was treated likewise in triplicate and activity determined. Red cell volume was then calculated as follows:

$$\frac{\text{Total radioactivity of blood injected}}{\text{(Activity per 100 ml. of 15 min. cells) minus (activity of initial cells)}} = \text{R.B.C. Volume}$$

Following a determination the animal was bled sufficiently to lower the hematocrit to the desired level and after allowing a few days for circulatory adjustment, another determination was made.

The red cell volumes as determined were then plotted against the corresponding jugular hematocrits and a straight line determined by the method of least squares was then drawn.

EXPERIMENTAL OBSERVATIONS. Although there is no loss of tagged red cells from the circulation by processes of elimination comparable to the loss of carbon monoxide or plasma volume dyes, nevertheless certain factors operate to require some caution in the analysis of data obtained by this method. If donor cells are injected into a normal dog, the usual circulatory equilibrium is altered and there follows a tendency toward reëstablishment of the former status. The result is that blood samples taken a day or so following the injection may show an increase in concentration of radioactivity in the red cells. This is due presumably to the fact that the animal has destroyed some of its own red cells. The cells from the donor animal are typical of red cells in an animal having had hypochromic anemia (by the nature of the preparation of the donor animals (6, 7)), as they are somewhat hypochromic and microcytic in nature, and are therefore somewhat more resistant than are the normal cells of the recipient animal.

We have also encountered another difficulty in the use of this procedure. It has not infrequently happened that the recipient dogs become "sensitized"

to the blood of the donor after repeated determinations are carried out. The symptoms are characteristic of a reaction of transfusion in dogs. There is marked discomfort, a fall in blood pressure, occasionally vomiting, a rapid thready pulse, running reflexes, and usually some spasticity of the hind legs. None of the reactions ended fatally in this series, but some animals were obviously very near death in several cases. Recovery is fairly rapid, usually requiring only about twenty minutes. Blood samples taken from such animals showed extremely marked hemolysis on centrifugation. All of the new isotopic cells had usually disappeared from the circulating cells within ten minutes of the time of injection of the donor cells. It is obviously impossible to determine the red cell volume of these dogs under these conditions, but the difficulty may be circumvented by use of another donor animal.

Discussion. Inspection of figure 1, charts A, B and C, indicates that there is a direct relationship, as is to be expected, between hematocrit and red blood cell mass in the sense that the increased cell mass is accompanied by increased

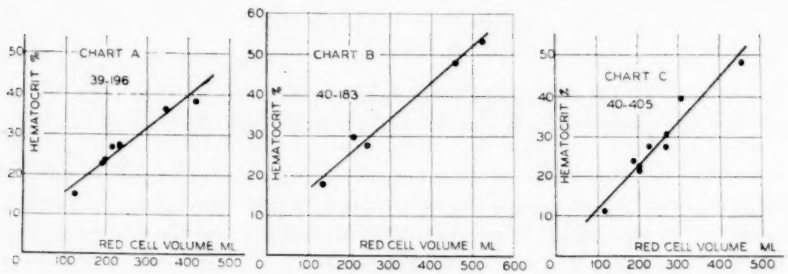

Fig. 1

hematocrit readings. As stated previously, the simplest formulation in this relationship would be:

$$\frac{\text{Hematocrit}}{\text{Red cell mass}} = \text{a constant}$$

Graphically this means that the experimental points should fall on a straight line passing through the origin. In dog 40-405, represented in chart C, the line obtained by the method of least squares is essentially of this character. In charts A and B, however, the straight line of best fit would indicate hematocrit values of 8 for zero cell mass. One suspects, therefore, that these points below a hematocrit of 10 should be fitted to a curve, rather than to a straight line. However, this region of the curve is not pertinent physiologically, since a dog cannot live with a hematocrit of much less than 10.

One may inquire as to the magnitude of the error resulting from the assumption that the red cell mass is proportional to the hematocrit. In chart B, assume that the circulating mass of red cells is known as 480 grams at a hematocrit of 50. By direct proportion at a hematocrit of 25 the red cell mass would be 240. Actually, as determined from the experimental curve, it is 200. The calculated value is thus in error by 20 per cent.

Elsewhere (3) it was shown that the total *circulating* blood volume remains constant with wide changes in venous hematocrit. Data presented here indicate that the cell volume is at least a linear function of hematocrit in the animals investigated. One would expect, therefore, that the circulating plasma value would be a decreasing function of the jugular hematocrit. Actually when one plots venous hematocrit against plasma volume, as determined by the Brilliant Vital Red dye dilution method (9), no close relationship can be demonstrated. This would suggest either that the plasma volume measured by this procedure is unreliable, or that the circulating plasma volume is not the same as the total plasma volume. We have suggested the latter in a recent communication (7). It is possible that the rapidly circulating plasma volume fraction would be inversely proportional to the hematocrit; but in order to prove this it will be necessary to determine this fraction independently of the red cell volume and not by simple calculation based on any assumption of a constancy between the jugular and average body hematocrit (6, 7). Perhaps this may be done by reinspection of the early portions of the curves of optical density of dye in the blood stream plotted against time. If the mixing time of the red cells is only slightly over two minutes (7) in the dog, it is quite possible that the mixing time of the dye in the rapidly circulating plasma fraction is of a similar order. There is no reason to think, in the absence of better evidence, that the rapidly circulating plasma volume is related in any consistent manner to the total plasma volume. Rather it is likely that the amount of plasma in either fraction is dependent on the state of the vascular system at any particular time and the proportion in each may well vary from one site to another simultaneously.

SUMMARY

By use of the donor-tagged cell method for the determination of red cell volume in dogs, it has been shown that in the individual dog a linear relationship exists between the red cell volume and the jugular hematocrit over a range of hematocrit of from 11 to 57 per cent.

It is suggested that the total plasma volume as determined by commonly employed methods is not necessarily representative of the actively circulating plasma volume.

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IDIOVENTRICULAR RHYTHMS AND FIBRILLATION INDUCED AT THE ANODE OR THE CATHODE BY DIRECT CURRENTS OF LONG DURATION

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The brief passage of direct current through the ventricle of the dog via non-polarizable electrodes has long been known to leave the tissues near the electrodes in a charged or polarized state. The discharging occurs gradually over a period of several seconds, rapidly at first and then more slowly, describing an exponential decay curve. Evidences have recently been presented which showed that the delayed responses to brief D.C. stimuli (*ca* 20 msec. duration) applied during the last half of local electrical systole were probably due to a persisting stimulus furnished by the charged state and the consequent polarization current. The response begins at the end of systole and consists of single or multiple premature systoles; sometimes a train of premature systoles leading into fibrillation (15). In order to learn more precisely the effects of weak prolonged constant currents on ventricular activity, and to further analyze the process of the initiation of fibrillation, experiments were designed in which polarity and rate of flow were known and varied at will, while the electrical activities of various areas of ventricular muscle were recorded.

PROCEDURES. *Animal preparations.* Twelve dogs, five cats and two monkeys were utilized in these experiments. The results reported are taken mainly from the experiments on dogs which are illustrative of the whole group. Dogs were anesthetized with morphine and sodium barbital, cats and monkeys with dial. The chests of the animals were opened by mid-sternal longitudinal incisions, and the hearts were suspended in pericardial cradles.

Recording and stimulating methods. Contiguous riding electrodes (10) led to three large Hindle string galvanometers which were arranged to record on the same 12 cm. paper. In all experiments records were made simultaneously from three local areas on the ventricle while a given test current was applied. The placement of the leads remained constant during all of the trials leading up to one fibrillation, but was varied from fibrillation to fibrillation to record the effects over the various parts of the ventricular surface with a maximum of thoroughness.

The apparatus for the application of the stimulating or polarizing current is shown diagrammatically in figure 1. The 110 volt direct current line, D.C., was connected across a voltage divider, V, which was used to regulate the voltage applied. In the circuit with the tissues was a 10,000 ohm series resistor, R_1, a milliammeter, A, with a scale reading 0 to 10, and a pole reversing switch, S_2. Contact with the tissues was made through Ag-AgCl electrodes, a large indifferent one under the skin of the upper end of the chest or of the forelimb, and a stigmatic one on the ventricle. The latter was about 1 mm. in diameter with

the contact end rounded to a bullet nose shape. This cardiac contact electrode was attached to a resilient multi-stranded copper wire in order that it might ride the ventricle, in the manner of the recording electrodes, without exerting undue pressure. The resistance of the tissue and electrode part of the circuit was approximately 1000 ohms. A calibrating circuit with a 1000 ohm resistor, R_2, was arranged so that by turning a selector switch, S_1, it could be substituted for the tissue circuit while the current was being adjusted before each test.

The current was allowed to flow for a maximum period of five seconds during each test. It was found experimentally that if fibrillation was to be produced by a current of given strength and polarity it almost always appeared during the first five seconds, usually the first three. The range of current intensities which were found useful was from about 0.5 to 5.0 milliamperes. At each current value records were made first with the cardiac electrode as the cathode, and two minutes later with this electrode as the anode. Neither the sequence of application nor the interval was observed to change the result. However,

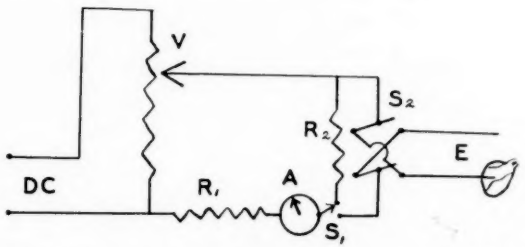

Fig. 1. Apparatus for applying direct current to the ventricle: *DC*, 110 volt source; *V*, 193 ohm voltage divider; R_1, 10,000 ohm resistor; R_2 1000 ohm resistor; S_1, selector switch; S_2, reversing switch; *A*, 0–10 milliammeter; *E*, Ag-AgCl electrodes.

the arrangement for the cathodal trial to come first, at each current strength, was based upon a good reason which will appear later.

Results. Examination of the beats left of the mark (x) in figure 2, A, B, C and D, shows the forms of electrograms recorded by contiguous electrodes during normally initiated beats. The record of a cycle consists essentially of a sharp initial spike and a T wave with an isoelectric line occupying the rest of the time, both systolic and diastolic. Polarizing currents of different strengths and directions cause changes of several kinds. For descriptive purposes we shall consider separately the effects of weak currents which cause no local discharges from the region of the electrode (subthreshold for premature beats), and of stronger currents which do induce local discharges (threshold or suprathreshold for premature beats).

The effects of *subthreshold currents* are illustrated in figure 2, A and B. The current was 2 ma. in both trials. The ventricular electrode (*S* on heart diagrams) was the cathode in A, and the anode in B. In these records, as in all others with subthreshold currents, the spikes show very little, if any, change in sharpness, width or direction. Spike height is also unchanged here, though

occasionally small changes may be seen. Large changes are evident, however, in the portions between spikes which were isoelectric in the absence of the polarizing current. Leads 1, 2 and 3 (see heart diagram) are recorded in order from

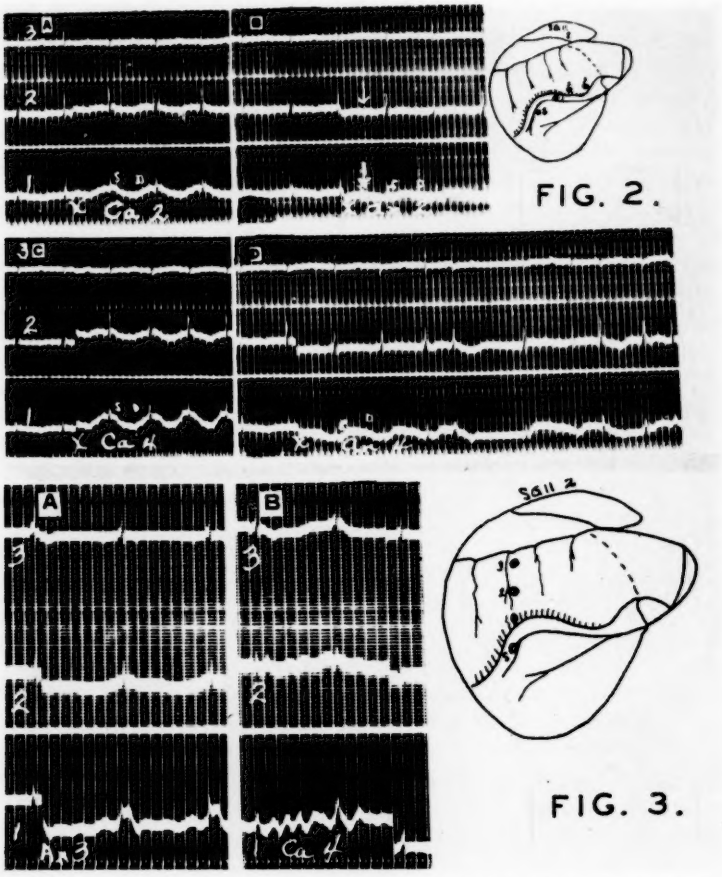

Fig. 2. Normal electrograms from contiguous electrodes and some effects of direct currents. Electrograms from points 1, 2 and 3 on heart diagram are recorded in order from bottom to top in all sections of figure. *X*, "on" shift of baseline when current was applied; *S*, moment of contraction of ventricle and *D*, relaxation; arrows, small oscillations (see fig. 3); vertical lines 40 msec. intervals in all records.

Fig. 3. A, Oscillations near anode; B, near cathode. In A, period is about 80 msec., in B it is 40 msec.

the bottom of the figure upward. In all leads there are "on" and "off" shifts of the base line when the circuit is closed (x) and opened (not shown). The magnitude of the shifts is influenced by the distance of the leads from the

polarizing electrode, their placement with respect to the lines of maximal current flow and the orientation of the two lead points. They are too small to be visible in L-3 of records A and B. In addition to the "on" and "off" shifts, two other changes are seen during the current flow: I. Inflections associated with the phases of muscular activity during the cardiac cycle (*S* and *D*), and II, oscillatory phenomena not temporally correlated with the muscular events (arrows).

The inflections associated with muscular activities are seen in leads 1 and 2. Here it is evident that in every case the systolic inflection (*S*) is in the direction of reducing the base line shift caused by the applied current, and, at the end of systole comes another inflection (*D*) returning the line to the position of maximum shift. In trying to explain these observations experiments were directed toward the detection and evaluation of artifacts which might be responsible for the deflections. The rocking of lead electrodes within the limits seen on a beating heart had repeatedly been shown in the absence of polarization to be without effect on the record. With polarization it was found that a degree of inclination of the electrode with respect to the cardiac surface very much greater than that seen in normal beats was required in order to produce a change in the recorded deflections, and then the most noticeable change was in the height of the spikes, indicating that at extreme inclination the contact was somewhat interfered with. Then the electrodes were supported so that they would only slightly indent the ventricle when relaxed. This made no differential changes in the systolic-diastolic deflections. Supporting the electrode to the extent of reducing the firmness of contact somewhat diminished all deflections including the spikes, the changes thus differing essentially from the phenomena under discussion. Holding the polarizing electrode so that it could not indent the relaxed ventricle was without observable effect. Some tests were made both with and without the 10,000 ohm current stabilizing resistor in the polarizing circuit, the necessary adjustments in voltage being made to keep the current at the desired values. The systolic-diastolic variations were very nearly equal with and without the resistor. This effectively rules out large cyclic changes in current flow as a cause of the deflections since the entire resistance of the electrodes and tissues is only one-tenth as great as the value of the constant resistor. With this resistor in the circuit large changes in tissue resistance could have only a relatively minor effect on the total resistance of the tissue circuit and, therefore, on the current flow.

The most probable explanation is that the systolic-diastolic variations in the recorded effect on baseline level of a constant current are due to changes in conductance in ventricular muscle, as distinguished from total conductance changes in all of the tissues and fluids contained in the circuit. Assuming that the galvanometer records represent potential differences between the two contiguous lead contacts, the direction of the changes is in agreement with the findings of Rapport and Ray (17) on conductance changes in the turtle heart. An increased conductance during systole, current flow being constant, would result in a lower potential difference between the two leads, and the converse

changes would occur during diastole. Suitable rotation of the lead electrodes minimizes base line shift and these deflections. The coincidence of these changes with contraction and relaxation rather than with excitation probably means that the changes in conductance are due to gross physical changes in the muscle and tissue fluids in the region of the pair of leads, not to cell membrane changes of the kind associated with excitation.

In addition to such systolic and diastolic deflections, record 2B exhibits rhythmic potentials with a period of about 40 msec., designated by arrows. Here they are small and require close inspection to be seen. In other arrangements of leads the phenomenon of oscillating potentials in the locality of the polarizing electrode was displayed with greater magnitude and clarity. Figure 3 shows them well. Here lead 1 was only about 7 mm. from the polarizing electrode and the three leads were aligned in the path of probable greatest current density, since the indifferent electrode was in the tissues near the neck end of the thoracic opening. The oscillations have a fundamental period of approximately 40 msec., or a frequency of 25 cycles per second. In many observations from dog and monkey hearts the intervals between crests have always been approximately multiples of 40 msec., i.e., 40, 80 and once 120 msec. In figure 3A the intervals are near 80 msec. with the anodal current of 3 ma., while in 3B the intervals are very close to 40 msec. with a cathodal current of 4 ma. When 4 ma. were applied anodally shortly thereafter there were mixed 40 and 80 msec. oscillations followed by fibrillation. Transitional periods were not seen. It appears reasonable to believe that these oscillations are the cardiac counterpart of the oscillating potentials seen in nerve placed in an environment low in Ca ions and cathodally polarized (1), or upon the mere removal of calcium (5). Oscillating potentials in nerve upon anodal polarization have not been reported, nor have frequencies forming a series of multiples been seen in nerve studies, but fundamentally the processes are probably similar.

Suprathreshold currents. When the intensity of the current is above the threshold for idioventricular discharges other changes become evident. Tachycardias and rhythms characteristic of each polarity of current occur. Figures 2C and D are samples. The deflections (*S*) and (*D*) between spikes remain qualitatively similar to those seen with weaker currents but accentuated. The anodal polarization threshold for local idioventricular discharges is about one and a half to two times as high as the cathodal. For example, in this experiment (fig. 2) the anodal thresholds were about 3.5 ma. and the cathodal 2.0 ma. A typical contrast in rhythms is shown in this figure. The cathodal record 2C shows a regular rate, but accelerated over that of the normally initiated beats by about 23 per cent. With stronger currents the increase may be as much as 40 per cent, the rhythm remaining regular. The anodal record 2D shows a coupled type of arrhythmia. The second discharge of each couple originates near the electrode and occurs within the T wave of the previous cycle whose excitation is of normal origin. This is one of the two most frequent types of arrhythmia seen upon anodal stimulation. The other type is shown in figure 4B and C. In this case there is an acceleration of beats until the interval

separating two spikes is of the duration of a normal electrical systole, i.e., a spike falls in the T wave of the preceding cycle. Then one of two things happens: *1*. There is a pause (*X*, *X*) after which begins another series of beats accelerating to the same limit as before, or (*2*), the accelerating paroxysm quickens its rate of acceleration and eventuates in fibrillation. Both of these sequences of events are seen in figure 4. After coupling also, a short accelerating paroxysm may occur and lead to fibrillation. Exceptionally, coupling and other arrhythmias are seen with cathodal stimulation. These rare responses seem to be more likely to occur in the cat experiments, though the cat responses ordinarily follow the patterns described.

Another feature often seen upon cathodal stimulation is a shifting in precedences of spikes recorded from two locations on the ventricle. The changes in time relations may be of relatively great magnitude. In figure 5A the spikes in leads 2 and 3 (middle and upper electrograms, respectively) show an alternating relationship. In the first beat after the current was applied lead 2 preceded lead 3 by 21 msec., then 3 preceded 2 by 36 msec., 2 led by 21 msec., 3 led by 24 msec., and similarly until the current was stopped. Upon looking at the spikes in only one of these electrograms the impression would be gained that long and short cardiac cycles were alternating, but the intervals are fairly constant between a given spike in lead 2 and the one in the next cycle in lead 3, from this to the next in lead 2, and to the next in lead 3, etc. These relationships suggest that the point of origin of the excitatory discharges is shifting. In the zone about the pole (*S*), the region of highest excitability probably is a ring around the electrode, and the discharge appears to originate in different parts of this peripolar zone, one time nearer lead 3 and next nearer lead 2. Distance, per se, may not be the only factor determining the relative times of excitation at these two points, but unequal accessibility of the two points because of changes in conduction may be a factor. The shifting origin of excitation is further attested by the alternating contours of the spikes in lead 1, indicating a different approach to this area every second beat.

Anodal polarization did not produce comparable shifting relationships between areas. In figure 5B the ventricular excitation is from the normal impulses, the 3 ma. current being subthreshold after the polarity was changed. In figure 4A, however, the cathodal lead 1 follows lead 2 by an interval that varies from 10 to 30 msec., while in the anodal records, B and C, 1 follows 2 by an interval which is much more constant, ranging from 17 to 21 msec.

Fibrillation. With constant current stimulation fibrillation is primarily an anodally induced phenomenon. A tabulation of all fibrillations in eleven consecutive animals showed 125 fibrillations resulting from anodal stimulation and only 5 from cathodal stimulation. More than 96 per cent of the fibrillations, therefore, were of anodal origin. In many animals 100 per cent of the fibrillations were anodal, and this is the finding to be expected when the current is carefully graded to be kept near the fibrillation threshold, though a very rare cathodal fibrillation will occur at relatively low threshold.

Fibrillation threshold must not be confused with the threshold for excitation

of local systoles. The two, however, are near the same in terms of current readings when anodal stimulation is used. Whenever local irregular discharges

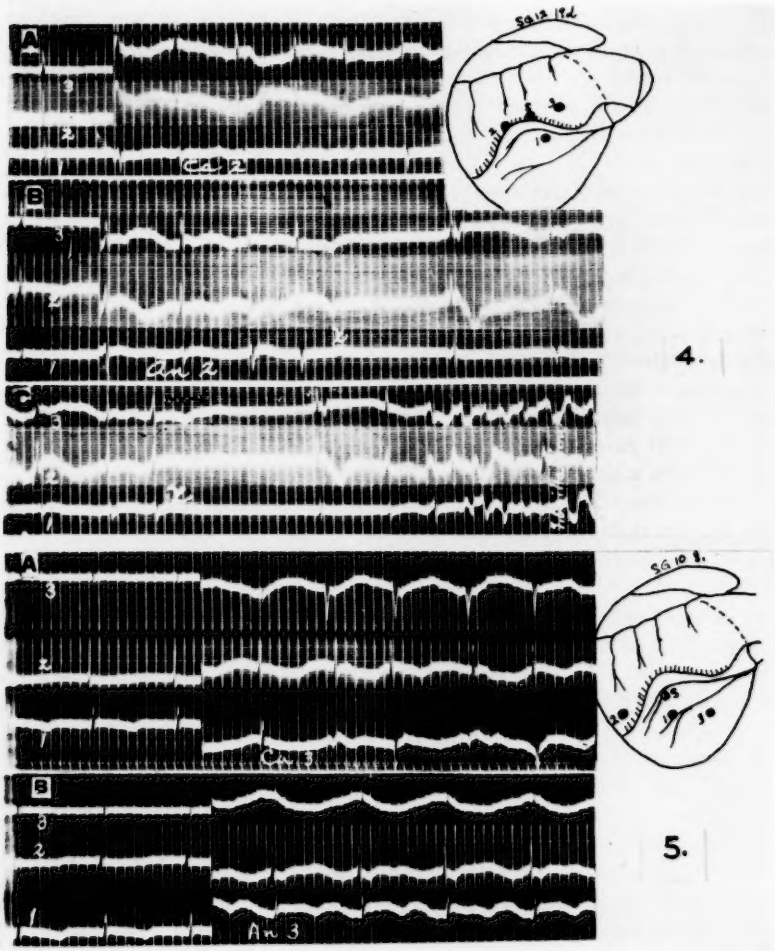

Fig. 4. Characteristic rhythms and fibrillation. A, 2 ma., cathode on ventricle. Local rhythm accelerated over normal rate, but with about normal regularity. B and C, 2 ma. anode on ventricle. Accelerating groups of beats interrupted by pauses at *X* and *X* followed by rapidly accelerating series eventuating in fibrillation.

Fig. 5. A, shifts in timing and sequence of spikes at points 2 and 3 with 3 ma. cathodal. Note changing shapes of spikes in L-1 (see text). B, 3 ma. anodal, subthreshold for local rhythms in this case.

or brief paroxysms of beats occur during anodal stimulation the probability is very great that fibrillation will occur within this application of three to five

seconds, or upon repetition of the same current a few minutes later. An increase of a few tenths of a milliampere may be required. With cathodal stimulation locally initiated beats and tachycardias offer practically no prospect of fibrillation. If, for example, local rhythms are induced by a current of 2 ma., fibrillation seldom results from a current of 5 ma., and cannot be depended upon to occur every time at 7 ma., though fibrillation usually will occur with such excessive cathodal currents.

The events leading to fibrillation with an anodal stimulus are shown in figure 6. Fibrillation is introduced by a brief series of premature beats, shown by the spike sequences to be originating in the region of the electrode. The intervals between these beats progressively diminish until the limiting interval of 80 to 90 msec. is reached and then fibrillation supervenes. At lead 1 fibrillation occurs after six premature beats, in lead two after seven or eight, and in

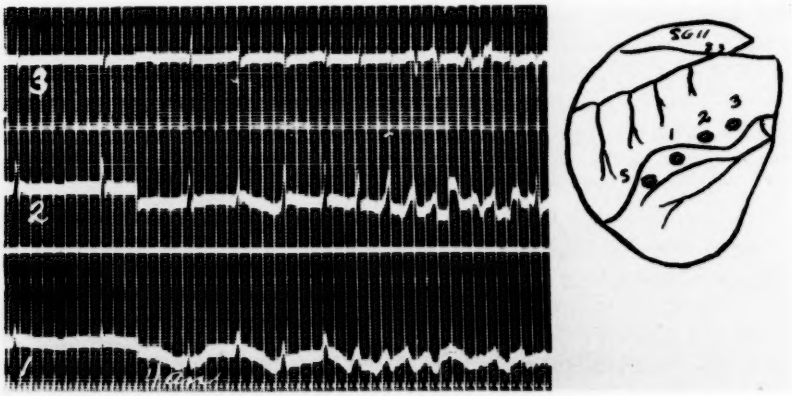

Fig. 6. Anodal fibrillation illustrating induction by discharges originating in region of polarizing electrode at accelerating rate.

lead 3 after eight or perhaps nine. Thus it is seen that the premature systoles and the fibrillation begin in the tissues adjacent to the electrode. The evidences which show that these premature deflections are due to discrete discharges and that they originate at the site of the electrode are the same as those described previously (15) found when fibrillation was evoked by a brief single stimulus placed in the latter part of systole. The train of discharges that introduces fibrillation upon anodal polarization resembles very closely the series that introduces fibrillation after a single brief stimulus. Fibrillation from a moderate (4 ma.) cathodal current occurs so seldom that one cannot say that any form is typical, but the cathodal series of discharges approaching fibrillation has been found to differ from the anodal approach in two ways: *1*. The cathodal series of rapid locally induced beats is long, containing about 20 spikes instead of 5 to 8. Such a long rapid bombardment has never been seen to precede an anodal fibrillation. *2*. Though there is an accelerating trend in this cathodal

series of rapid beats, there is some slight waxing and waning in frequency as the general overall acceleration takes place.

Summary of relationships between the effects near the anode and near the cathode when galvanic currents are applied to the mammalian ventricle

EFFECT	POLARITY	
	Anodal	Cathodal
Production of systolic-diastolic shifts in base line	No difference except that directions are opposite	
Production of oscillating potentials	Lower waves, longer periods; (80 msec.) likely	Higher waves. Basic periods of 40 msec. likely
Tachycardia or local discharge threshold	Higher by 1½ to 2 times	Lower
Rhythm of tachycardia	Characteristically irregular	Usually regular
Relation between times of onset of spikes at given separate points	Relatively constant	Shifts in timing and sequence
Probability of fibrillation when local discharges are induced	Great	Small

DISCUSSION. *Fibrillation: A result of effects of local repetitive activity.* The elucidation of the mechanism of the initiation of ventricular fibrillation, and the discovery of agents and procedures which facilitate or inhibit its development constitute the objective of the series of electrographic studies of which this one is a part. Moe, Harris and Wiggers (15) recently demonstrated that the fibrillation which results from a brief electrical stimulus applied late in systole is preceded by a train of locally initiated beats occurring at an accelerating rate. The present experiments show that the fibrillation that results from the application of constant current is introduced by a similar accelerating series of discharges from the region of the polarizing electrode. From these and other findings in these two studies it may be deduced that this rapid accelerating activity brings about changes in the functioning of the myocardium which allow the intervention of fibrillation. It has long been known that as the heart rate increases electrical systole shortens, the refractory period shortens, and the rate of conduction diminishes (14). Moe, Harris and Wiggers (15) observed upon the application of induction shocks to mammalian ventricles in a serial manner with progressively diminishing intervals of separation that the refractory periods progressively diminished and conduction was slowed in succeeding systoles. Fibrillation was produced if the rhythm of the applied shocks was made to accurately mimic the rhythm of the discharges leading to fibrillation after a brief stimulus. Slowed conduction coupled with a short refractory period are conditions which, sufficiently developed, permit the occurrence of circuitous reëxcitation or fibrillation.

Anodal and cathodal polarization. The salient observation in this study of fibrillation by constant currents is that of the pre-eminence of anodal polarization as a fibrillating agent, and, by contrast, the relative impotence of cathodal polarization. However, in the production of locally initiated impulses and tachycardia weak currents are more effective at the cathode than at the anode. The processes involved in the establishment of fibrillation, therefore, must contain some factor or factors which differ significantly from those necessary for mere local initiation of impulses, though these are a prerequisite to fibrillation.

Changes in properties related to excitation which attend anodal polarization, other than the classical electrotonus phenomena, are the development of recovery through supernormality (4) and slowing of accommodation (19).

Supernormality and fibrillation. Ashman and Hafkesbring (2) attributed intermittent groups of discharges in turtle heart muscle, initiated by an induction shock, to recovery through supernormality. Treppe and fatigue with attendant increases and decreases in local excitability were offered in explanation of the observed waxing and waning, respectively, in the frequency of discharge during a group, and the further development of fatigue led to the intervals between groups. In our polarization experiments, however, rhythms which closely resembled those of Ashman and Hafkesbring were never obtained. The idioventricular beats which occurred upon *cathodal* polarization were usually found to be quite regular in rate. An exception is the rare case of fibrillation at the cathode with moderate current. A relatively mild intermittence is evident in the long accelerating train of beats. During *anodal* polarization intermittence often occurred, but not with waxing and waning in frequency of discharge. The usual pattern is acceleration throughout the period. If the period of idioventricular impulses ended without fibrillation the end came when the intervals between discharges became reduced until the R spike of one electrogram reached the T wave of the preceding one. The time relation between the end of one systole and the beginning of the next one in such a series of beats was progressively changing from one beat to the next, the two approximating each other more closely with each succeeding cycle. This systematically and rapidly changing relationship is hardly consistent with the view that the discharges result from recovery through supernormality. Many experiments by Moe (unpublished) in which the cardiac cycle was minutely scanned by testing stimuli failed to reveal evidence of a supernormal period in the ventricles (unpolarized) of open chested dogs. A few trials made by us during the flow of weak polarizing currents were negative also, but on account of the smallness of the number of trials and certain technical inadequacies, these were considered inconclusive.

In favor of the view that supernormality could have occurred is the finding of Hoff and Nahum (12) that supernormal excitation occurs in mammalian ventricles under certain experimental conditions and the demonstration of Blair (4) that in nerve anodal polarization produces the property of recovery through supernormality. The coupling type of arrhythmia occasionally seen may reasonably be accounted for as a manifestation of recovery from the first beat

of each couple, usually of normal origin, through supernormality. The second beat in the couple was of local origin and occurred during the latter part of the T wave or shortly thereafter, corresponding very well with the statement of Hoff and Nahum that supernormality occurs during the U wave. However, coupling is not the kind of response which introduces fibrillation, and the evidence at hand does not indicate that supernormality plays an important rôle in its production.

Accommodation and fibrillation. Evidences from nerve physiology reveal that a readiness for spontaneous activity, or repetitive discharging in response to a constant stimulus or a single brief stimulus is regularly accompanied by a low rate of accommodation (13). Erlanger and Blair (8) found that sensory nerve fibers repeat more readily than do motor fibers, and correspondingly, sensory fibers accommodate less rapidly than do motor fibers. They have also observed that anodal polarization enhances or produces repetition in excised frog nerve subjected to a constant stimulus (7). Mammalian nerves with circulation intact respond to slightly super-rheobasic currents with repetitive discharges at the cathode or anode, the greater series being elicitable at the anode (18). Accommodation is slow in circulated nerves (20) (16), and anodal polarization has the effect of reducing accommodation while cathodal polarization increases it (19).

Under conditions of slow accommodation, according to B. Katz (13), a single strong brief stimulus applied to a nerve evokes a multiple response in its fibers. In general, the slower the process of accommodation the longer the period of repetitive discharge from a stimulus of given strength. Likewise, the more intense the stimulus (the higher the "local potential," Hill (11)) accommodation being equal, the longer the period of repetition. If the rate of accommodation is sufficiently slow the "local potential" may remain above the threshold during a period long enough to span many discharges and their refractory periods.

If anodal polarization reduces the rate of accommodation in cardiac muscle as it does in nerve, and if cathodal polarization correspondingly increases accommodation, a reasonable explanation is available for the great effectiveness of anodal polarization as a fibrillating agent and for the relative ineffectiveness of currents at the cathode in the production of fibrillation.

Local responses in tissues which exhibit an autogenous excitability cycle like cardiac muscle may be classed in two categories: first, responses in which there is one discharge only upon each local rise of the excitatory process, and second, multiple discharges from a single rise of excitability. In the first group would be the ventricular responses seen upon cathodal polarization and the anodal responses which are spaced more widely than the duration of a normal electrical systole. The regularity of the discharges and the increase in rate above the normal during the tachycardia from a cathodally polarized zone are indicative of a uniformly elevated excitability suggesting the plateau in the cathodal excitability curves found in heart experiments by Gilson and Peugnet (9) and in nerve experiments by Erlanger and Blair (6). This plateau represents a state of balance between two processes, one enhancing irritability ("local potential")

and the other depressing it (accommodation). This regularity of discharge observed during cathodal tachycardias practically eliminates the possibility that any multiple discharges resulted from any single rise of the local excitatory process during these trials. An absence of multiple responses to a single excitation would be a logical result of rapid accommodation. Sufficiently excessive suprathreshold currents, conceivably, could over-ride the accommodation and produce a group of repetitive discharges from a single local cyclic excitation and thus quickly produce the great acceleration of heart rate required for the induction of fibrillation. Excessive currents did sometimes produce such results at the cathode.

In their study of repetitive responses to polarizing currents, Erlanger and Blair (7) found that threshold curves of nerve fibers at the cathode do not always describe a smooth plateau. Those yielding different types of repetitive responses exhibited correspondingly different threshold curves, i.e., rising, falling or oscillating thresholds. A gradually falling threshold at the cathode on the ventricle could explain the gradual acceleration seen on those rare occasions when such acceleration and eventual fibrillation resulted from moderate cathodal stimulation.

In multiple responses resulting from a persisting excitatory state following a single stimulus, autogenous or applied, each discharge should occur immediately upon the restoration of normal excitability. Moe, Harris and Wiggers produced evidence which indicates that this restoration is complete at the end of the T wave. No reduction in threshold for extrasystoles was demonstrable after this, nor was there further shortening of the latent period of extrasystoles evoked by brief stimuli. Discharges which are due to a persisting local excitatory state, therefore, may be expected to occur at or before the end of the T wave. Those which occur appreciably later must be due to a new stimulus. The characteristic short series of about five to seven discharges occurring at short and diminishing intervals which leads to fibrillation upon anodal polarization abundantly obeys the condition prescribed for multiple discharges from a single stimulus. In every case succeeding spikes in these runs of beats have fallen within an interval shorter than the R-T of a normal cycle, and when the limiting interval between spikes of about 80 msec. is reached it is less than half the duration of the R-T segment of a normal electrogram (170–210 msec.). Each time that fibrillation from anodal polarization occurs the same pattern of accelerating discharges is almost repeated, and this typical pattern again is very similar to that which introduces the fibrillation that results from a single brief stimulus. This essential similarity of patterns of series of beats leading to fibrillation, one excited by a single brief stimulus and the other by the anode of a constant current considered together with the fact that anodal polarization reduces accommodation and therefore conveys the capability of multiple responses to a single excitation present a forcible argument that the anodal series is also a multiple response to a single excitatory process arising within the tissue. The essential condition for facilitating the production of fibrillation appears to be a low rate of accommodation.

Other direct effects at the anode, reported in nerve studies, which may well be factors contributing to the establishment of fibrillation are local slowing of conduction and shortening of the refractory period (3). These changes are qualitatively like those produced in the whole ventricle by a rapid series of discharges, however excited. It might be argued that the direct effects of the current at the anode, slowing conduction and shortening refractoriness, constitute the prime factor in the induction of fibrillation. But the well established observations *1*, that each fibrillation is preceded by an accelerating series of local discharges, never occurring without them, and *2*, that fibrillation is occasionally produced at the cathode without excessive current may be regarded as evidence that the rapid discharges are of main importance in the production of the changes that permit fibrillation. The direct anodal effects of slowing conduction and shortening refractoriness probably play a contributing rôle, though its importance cannot at present be estimated. The indispensable factor for the production of ventricular fibrillation seems to be a very rapid series of locally excited beats.

SUMMARY

A method for applying currents controlled as to polarity and rate of flow to the mammalian ventricle is described. Responses from practically the whole ventricular surface were sampled by recording from three local areas simultaneously and changing the positions of the riding contiguous electrode leads from time to time.

Currents, subthreshold for extrasystoles, could give rise to several kinds of changes in the record: *1*, "on" and "off" shifts of the base line upon closing and opening the circuit respectively; *2*, inflections and changes of level associated with the phases of muscular activity. The amount of displacement of the base-line was reduced during systole, restored to maximal during diastole. After ruling out contact artifacts these features were attributed to conductivity changes associated with contraction and relaxation.

Both anodal and cathodal polarization give rise to oscillating potentials not associated with the phases of the cardiac cycle. The periods of the oscillations were near 40 and 80 msec. Cathodally produced oscillations are higher and are likely to be of the higher frequency (25 cycles per sec., 40 msec. period).

The idioventricular beat threshold was lower for cathodal polarization than for anodal, but the anodal threshold for fibrillation is the lower. The anode is preëminently effective in producing fibrillation. With equal applications of currents near fibrillation threshold 96 per cent of all fibrillations were of anodal origin.

Cathodal polarization of suitable intensity usually produces a tachycardia of regularly spaced idioventricular beats, while anodal polarization produces characteristically irregular rhythms. The point of origin of the discharges appears to be more constant in anodal polarization. In all cases of fibrillation resulting from polarizing currents induction is via an accelerating series of discharges from the region of the polarizing electrode.

In the discussion, findings from studies on excitation and spontaneous rhythmicity in nerve and muscle are considered and correlated with the observations on fibrillation. Absence of, or a low rate of accommodation appears to be the condition essential to spontaneous rhythmicity and multiple discharges in response to a single excitation. The probable association of the reduction of accommodation by anodal polarization with the great effectiveness of the anode in the production of fibrillation is pointed out. Direct anodal effects of locally reducing the conduction rate and reducing the refractory period are considered and assigned a contributing rôle.

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THE ACTION OF IONS ON THE MAMMALIAN HEART

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There have been few studies of the effects produced on the heart by varying the concentration of Ca, K, Na and H ions within limits close to normal. This is especially true of the mammalian heart. The present study was made to obtain further information concerning the effect on various mammalian heart preparations of varying the concentration of the plasma ions within these limits.

EXPERIMENTAL. Three different types of preparations were used in these experiments. The right atrium preparation of the guinea pig (Spealman, 1940) was used to study the effects of the ions on the rate of beat, the amplitude of contraction, and the duration of contraction of the heart. The term "duration of contraction" is used to mean the total period during which the heart muscle is under increased tension. In these experiments, the procedure was to perfuse the heart with normal Locke's solution for a period of from 20 to 40 minutes; then the experimental Locke's solution which was to be studied was perfused for a similar length of time; and finally, normal Locke's solution was again perfused.

Values for the heart rate, the amplitude of contraction, or the duration of contraction as the case might be, were recorded every 10 or 15 minutes. The heart rate was determined, using a stop watch. The amplitude of contraction was determined by noting the extent of excursion of the heart lever on a millimeter ruler held close to the lever but not touching it. The duration of contraction was determined from kymographic tracings of the heart lever.

The Langendorff preparation was used for electrocardiographic studies on the excised guinea-pig heart. Just as in the experiments with the right atrium preparation, records were taken with the heart perfused with normal Locke's solution both before and after the observations with the experimental Locke's solution. The electrodes used for contact with the heart were silver wire coated with silver chloride. Contact was made by inserting one electrode through the atrial tissue and the other through the ventricular tissue. Only the effects of varying the Ca and K ion concentrations were studied in these experiments.

Dogs anesthetized with Dial or Nembutal were used for observations of the effect of Ca and K ions on the heart in the intact animal. The procedure was to infuse isotonic concentrations of these salts intravenously at the rate of 1 to 3 cc. per minute over a period of an hour or more in order to increase the concentration of either of these salts. Experimental lowering of the serum Ca was accomplished by infusing isotonic sodium oxalate at the rate of 1 to 2 cc. per minute. Lowering of the serum K was brought about by a short (10 min.) intravenous infusion of 1 in 20,000 epinephrine at a rate of 1 cc. per minute. As D'Silva (1934) and others have shown, this causes a rather prolonged lowering of the serum K; so it was possible in the present experiments to allow the

effect of epinephrine to disappear before taking observations. In most of these experiments (see tables 3 and 4) the vagi were cut; for evidence in the literature indicates that the vagus center might be strongly stimulated by infusing $CaCl_2$ (Hoff et al., 1939) and possibly by KCl. Electrocardiographic observations

TABLE 1

The rate of beat, the amplitude of contraction, and the duration of contraction of the guinea-pig right-atrium when perfused with Locke's solution of various compositions

In each experiment the average of the values obtained with normal Locke's solution was taken as 1.00. The results of several experiments are averaged to give the values in the table. The letters P.D. signify that a progressive decrease in heart rate or in amplitude occurred which prevents the calculation of a numerical value in these cases. — indicates that no data were obtained at that concentration.

a. Ca ion concentration of Locke's solution varied

	Ca ION CONCENTRATION (M/L)					NUMBER OF HEARTS
	0.0005	0.001	0.002	0.004	0.008	
Rate	0.86	0.98	1.00	1.02	P.D.	3
Amplitude	0.12	0.46	1.00	1.50	P.D.	3
Duration	—	0.97	1.00	1.17	—	2

b. K ion concentration of Locke's solution varied

	K ION CONCENTRATION (M/L)				NUMBER OF HEARTS
	0.001	0.002	0.004	0.008	
Rate	P.D.	0.98	1.00	0.85	2
Amplitude	0.77	0.97	1.00	0.53	2
Duration	—	1.22	1.00	1.02	4

c. Na ion concentration of Locke's solution varied

	Na ION CONCENTRATION (M/L)					NUMBER OF HEARTS
	0.10	0.14	0.15	0.16	0.20	
Rate	1.00	0.97	1.00	0.99	0.88	2
Amplitude	0.64	1.01	1.00	0.97	0.29	2

d. H ion concentration of Locke's solution varied

	H ION CONCENTRATION (pH)					NUMBER OF HEARTS
	7.0	7.4	7.5	7.6	7.7	
Amplitude	1.01	1.02	1.00	—	1.05	2
Duration	—	1.04	1.00	0.98	—	2

(lead II), observations of the intraventricular pressure with the Hamilton manometer, and blood samples were taken at approximately half-hourly intervals. The sera of the blood samples were analyzed for K by the method of Kramer and Tisdall (1921a) or for Ca by the method of Kramer and Tisdall

(1921b). Since only part of the serum Ca exists as Ca ion, the serum Ca determinations only indicate the changes of Ca ion concentrations which took place in the experiments.

TABLE 2

The electrocardiogram of the guinea pig heart perfused by the Langendorff method with Locke's solution of different Ca and K concentrations

In each experiment the average of the values found in normal Locke's solution was taken as 1.00. In certain cases the results of several experiments are averaged to give the values in the table (see under remarks).

a. K ion concentration of Locke's solution varied

K ION M/L	R-R	P-R	Q-T	REMARKS
0.003	1.00	1.11	0.91	Average for 2 hearts—2 to 1 block occurred in one
0.004	1.00	1.00	1.00	Normal Locke's solution
0.006	1.03	0.89	1.04	Average for 4 hearts
0.007	1.18	0.87	1.04	One heart, progressive decrease in rate occurred

b. Ca ion concentration of Locke's solution varied

Ca ION M/L	R-R	P-R	Q-T	REMARKS
0.001	1.20	0.75	1.90	One heart, progressive decrease in rate occurred
0.002	1.05	0.90	1.34	Average for 2 hearts
0.004	1.00	1.00	1.00	Normal Locke's solution
0.006	1.06	1.17	1.05	Average for 2 hearts. Occasional block occurred in both

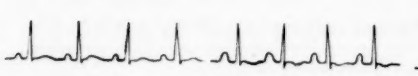

Fig. 1

Fig. 2

Fig. 1. The original electrocardiograms were redrawn as accurately as possible on graph paper to obtain the above reproductions, which illustrate the usual changes occurring in the present experiments during intravenous infusion of $CaCl_2$. In the record to the left (control), the serum Ca was 2.4 mM/L; in the record to the right (taken 105 min. later), the serum Ca was 4.2 mM/L. Note the increase in the P-R interval and in the height of P-wave in record to the right.

Fig. 2. The above reproductions, obtained as in figure 1, illustrate the usual changes in the present experiments occurring during intravenous infusion of KCl. In the record to the left (control), the serum K was 4.3 mM/L; in the other record (taken 85 min. later) the serum K was 6.2 mM/L. Note the decrease in the P-R interval and the increase in the height of the T-wave in the record to the right.

RESULTS. Table 1 summarizes the results obtained on the right atrium of the guinea pig. All values in the table are expressed as decimal fractions of the average of the values obtained with the normal Locke's solution. The table

shows that, within limits close to normal, *1*, the heart rate is uninfluenced by variation of the concentrations of Ca, K, or Na ions; *2*, the amplitude of contraction increases as the Ca ion concentration increases but is not greatly affected by the other ions studied, and *3*, the duration of response is slightly influenced by both Ca and K ions but not by H ion. The table further shows that depressive changes or abnormalities such as a decrease in the heart rate or a depression of the amplitude of contraction, may appear when the concentration of these ions is too different from normal. No depressive changes were found with H ion; this is probably because the concentration was not varied sufficiently.

Table 2 summarizes the electrocardiographic data obtained on the guinea-pig heart perfused by the Langendorff method. All values are expressed as decimal fractions of the average of the values obtained with normal Locke's solution. The data suggest that the R-R interval is affected in these experiments only when the perfusion solution was sufficiently abnormal to cause a lengthening of this interval. The P-R interval decreases as the K ion concentration rises, and increases as the Ca ion rises. The Q-T interval appears not to be greatly

Fig. 3. Intraventricular pressure records illustrating the increase in systolic pressure that usually occurred during intravenous infusion of $CaCl_2$. In the record at the left (control), the serum Ca was 2.4 mM/L; in the middle record (taken 30 min. later), the serum Ca was 4.7 mM/L; and in the record to the right (taken 35 min. after the middle record), the serum Ca was 6.6 mM/L. The pressure values obtained from the above records are respectively 135, 180 and 255 mm Hg. Time 0.2 sec.

affected by K ion concentration, but to be lengthened by a lowering of the Ca ion concentration.

Figures 1, 2 and 3 illustrate some of the positive findings in the electrocardiographic and intracardiac pressure studies in acute experiments with intact animals (dogs). Figure 1 shows that the P-R interval and the height of the P-wave are increased with increasing serum Ca concentration. Figure 2 shows that the P-R interval is decreased and the height of the T-wave is increased with increasing serum K concentration. Figure 3 shows that the intraventricular pressure is increased with increasing serum Ca concentration. A summary of the total findings in these experiments is given in tables 3 and 4. The tables show that the effects illustrated by the figures were present in most of the dogs studied. These tables also give information on other measurements which will be discussed below.

Discussion. A difficulty arises in the interpretation of the data obtained in this study. The effect produced when the concentration of any cation of Locke's solution or of the plasma is changed may be a true "physiologic" effect or it may

be an unspecific effect which only signifies that the perfusing fluid is abnormal. This fact must be kept in mind especially when the results in tables 1 and 2 are considered.

In the following discussion, the various activities or properties of the heart are considered under separate headings.

Heart rate. The rate of beat of the guinea pig right atrium is not affected by varying the concentration of the Na, K, Ca or H ion, provided these are within limits close to normal. Outside certain limits, the heart rate tends to decrease. This is likewise true of the Langendorff guinea-pig preparation for the ions (Ca

TABLE 3

Changes in the systolic intraventricular pressure (I.V.P.), in the duration of the intraventricular pressure curve (Dur.), and in the electrocardiogram (lead II) in the intact dog during experimental variation of the plasma Ca concentration

The number of dogs studied and the average maximum change in the serum Ca are indicated in the first two columns. The table indicates the number of dogs which showed an increase (+), no change (0), or a decrease (−) in the various values at the highest or lowest experimental values reached. Occasionally accurate readings of some of the values could not be obtained; in these cases fewer observations are recorded than there were experimental animals. The vagi were cut in all animals.

NO. OF DOGS	CHANGE IN SERUM Ca, MM/L	HEART RATE			I.V.P.			DUR.			P-R			Q-T			HT. OF P			HT. OF R			HT. OF T		
		+	0	−	+	0	−	+	0	−	+	0	−	+	0	−	+	0	−	+	0	−	+	0	−
5	+2.94	5	0	0	4	1	0	0	0	5	4	0	1	0	1	2	3	0	0	1	0	4	2	1	2
2	−0.90	0	1	1	0	0	2	1	1	0	0	0	2	1	0	0	0	0	2	0	0	2	1	0	1

TABLE 4

Cardiac changes occurring during experimental variation of the plasma K concentration

This table is constructed in the same manner as table 3. The vagi were cut in all except one animal in each group.

NO. OF DOGS	CHANGE IN SERUM K, MM/L	HEART RATE			I.V.P.			DUR.			P-R			Q-T			HT. OF P			HT. OF R			HT. OF T		
		+	0	−	+	0	−	+	0	−	+	0	−	+	0	−	+	0	−	+	0	−	+	0	−
6	+1.71	3	2	1	4	2	0	0	0	6	0	0	6	1	1	3	2	3	0	1	3	2	4	2	0
3	−0.90	1	1	1	0	0	3	1	0	2	2	1	0	0	2	1	2	1	0	2	0	1	0	1	2

and K) studied. There is no general agreement in the published literature concerning the physiologic effects of the various ions on the heart rate; and the present findings give no support to the positive findings of others. In the acute experiments on the dog, the heart rate showed a tendency to increase, especially with the infusion of calcium chloride. In this case, the average increase was 25 beats per minute. Others have reported a decrease in heart rate as a result of infusing calcium chloride, supposedly due to vagus stimulation (Hoff et al., 1939); however, in the present experiments the vagi were cut. Possibly, this acceleration in heart rate is due to sympathetic stimulation; however, the heart

rate often remained increased even after infusion was stopped and the serum Ca was approaching its original value.

Amplitude of contraction. Ca ion seems to be the chief cation which is believed to affect the amplitude of contraction of the heart. However, there are several reports in the literature indicating that K ion affects the amplitude of contraction in a manner opposite to Ca ion. In the present experiments there was a marked increase in amplitude of contraction of the guinea-pig heart as the Ca ion concentration was increased. Both Na and K ions, when in sufficiently high or low concentration, caused a depression of the amplitude of contraction, which is presumably due to the abnormality of the solutions. In the acute experiments on the dog, variation of the plasma K concentration caused some change in the intraventricular systolic pressure; however, this was small. With increased serum K, there was an average increase in blood pressure of 7 mm. Hg; with decreased serum K, there was an average decrease of 15 mm. Hg. If this slight effect is significant, it should be noted that it is in the opposite direction to that usually postulated for K ion on the excised heart. However, variation of the serum Ca concentration caused marked changes in the intraventricular pressure. The average increase with increased serum Ca was 36 mm. Hg and the average decrease with decreased serum Ca was 65 mm Hg. This effect is in agreement with the fact that Ca ion increases the amplitude of cardiac contraction. It must be admitted, however, that this effect of Ca ion on the intracardiac pressure in the intact animal might not be a direct effect of Ca ion on the heart. A definite conclusion concerning the site of action of Ca ion in these experiments cannot be obtained from the present data.

Duration of ventricular response. Since the time of Ringer, it is generally believed that increasing the K ion concentration causes a decrease in the duration of the ventricular response, while increasing the Ca ion concentration increases the duration of the response. The present findings on the guinea-pig heart tend to support the above belief; however, the changes found are of small magnitude. The results obtained on the heart in the dog experiments are difficult to interpret owing to the fact that the heart rate often increased appreciably. This may be the explanation of the slight shortening of the duration of the intracardiac pressure curves in some of the experiments.

Electrocardiographic changes. In the following discussion it should be kept in mind that the heart rate and blood pressure showed some variations in the acute experiments with the dog, especially in the case of infusion of calcium chloride. Possibly some of the changes in the electrocardiogram are secondary to these changes.

The studies on the Langendorff preparation of the guinea-pig heart indicate that the P-R interval is decreased by increasing the K ion concentration, and increased by increasing the Ca ion concentration. The experimental findings on the heart in the intact dog are in agreement with those on the perfused guinea pig heart, so far as the P-R interval is concerned. Observations in the literature on the effects of Ca and K ions on the P-R interval are rather few and cannot be discussed here.

Other effects of Ca and K ion on the electrocardiogram were noted. On the excised guinea-pig heart, it was found that the Q-T interval was considerably lengthened when perfused with Locke's solution containing low Ca ion concentrations. This finding is of interest since this alteration of the electrocardiogram has been reported in cases of hypocalcemia in man (Carter and Andrus, 1922). The evidence that the Q-T interval is influenced by the plasma Ca concentration is only suggestive in the case of the present dog experiments.

On the heart of the dog it was further found that the height of the P-wave tended to vary in the same direction as the plasma Ca concentration. The electrocardiographic results obtained on the guinea-pig heart do not show this effect. However, Edwards and Page (1926) found that dogs treated with parathyroid extract sometimes showed an increased P-wave amplitude. The other electrocardiographic results obtained with the intact dog showed no significant variation with the exception that the height of the T-wave showed a tendency to vary in the same direction as the plasma K concentration. This is of interest since an increased amplitude of the T-wave due to increased K concentration has been reported (Nicholson and Schecter, 1937).

SUMMARY AND CONCLUSIONS

1. In the guinea-pig right atrium preparation, certain depressive or abnormal changes, such as a decrease in rate which was usually progressive, a depression of the amplitude of contraction, or arrhythmia, occurred when the concentration of the various ions was too different from normal. Within limits close to normal, the most definite positive effect was the variation of the amplitude of contraction with the Ca ion concentration. There was also some suggestion that increasing the Ca ion concentration caused an increase in the duration of the response, while increasing the K ion concentration caused a decrease in the duration of the response.

2. In the Langendorff preparation of the guinea pig heart, the heart rate was independent of the K ion and Ca ion concentrations within regions close to normal, but was depressed in certain instances where the concentration was too different from normal. The P-R interval lengthened as the Ca ion concentration increased, and shortened as the K ion concentration increased. The Q-T interval was lengthened as the Ca ion concentration decreased, but was not greatly affected by changing the K ion concentration.

3. In the acute experiments on dogs, in which the plasma Ca concentration was varied, the intraventricular pressure, the P-R interval, and the height of the P-wave all tended to vary in the same sense with the plasma Ca concentration. The duration of response was slightly decreased and the heart rate was increased when the plasma Ca concentration was raised. The Q-T interval, the height of the R-wave, and the height of the T-wave showed no very definite tendencies to vary with the plasma Ca concentration.

4. In the acute experiments on dogs in which the plasma K concentration was varied, the intraventricular pressure and the height of the T-wave tended to vary in the same sense and the P-R interval in the opposite sense with the plasma

K concentration. The magnitude of the intraventricular pressure changes was small. The duration of response was slightly decreased when the plasma K concentration was raised. The heart rate, the Q-T interval, the height of the P wave, and the height of the R wave showed no definite tendencies to vary with the plasma K concentration.

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THE INFLUENCE OF BILE SALTS ON ACTIVE INTESTINAL ABSORPTION OF CHLORIDE

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Active intestinal absorption of chloride against concentration gradients has been extensively studied in recent years by Ingraham and Visscher (1, 2, 3), who have shown that under certain experimental conditions the lower ileum can reduce the chloride concentration in the lumen to values as low as 0.5 per cent of the blood plasma level. It appears probable that the active process involved is responsible for a large share of the chloride absorbed by the intestine. In view of the importance of bile salts in the absorption of a wide variety of substances, it seemed desirable to study their influence on active chloride absorption.

METHODS. Dogs were anesthetized with pentobarbital sodium injected intraperitoneally. A loop of lower ileum about 2 feet long and about 6 inches from the cecum was isolated. A rubber cannula was inserted in one end of the loop and a bent glass cannula in the other. It was then rinsed out with 3 to 4 liters of isotonic NaCl at about 37° until the washings were clear. A rubber cannula was then substituted for the glass one, and the loop was again rinsed out to remove traces of blood. After the rubber cannulas had been closed with short lengths of glass rod the loop was returned to the abdominal cavity for a few minutes. It was then drawn out and divided into two adjacent loops of equal length by a ligature placed at its center. The loops were returned to the abdominal cavity and allowed to rest for 30 minutes. The dog was kept warm with an electric lamp during the entire experiment.

At the end of the rest period a control solution of half isotonic sodium chloride and half isotonic sodium sulfate at 37° was injected into one loop, and a similar solution containing bile salts was injected into the other. Fifteen minutes were allowed for absorption. The loops were emptied into graduates, and after the volume was read, each was washed out rapidly with 15 cc. of distilled water. The recovered fluid and washings in each case were made up to 50 cc. and analyzed for chloride by the method of Van Slyke (4).

In a large number of experiments the bile salt solutions used had sulfate and chloride concentrations equal or close to those of the controls, but in many cases the distilled water, added to maintain isotonicity, lowered these values considerably. In calculating isotonicity 50 per cent ionization of the bile salts was assumed except in the case of sodium glycocholate in which the calculation was based on the data of Roepke and Mason (5). The sodium chloride present as an impurity in the commercial bile salt, sodium taurocholate, and one of the sodium glycocholate preparations was also considered in estimating isotonicity.

Other experiments were carried out with sodium acetate, glucose, sodium bi-

TABLE 1

Influence of commercial bile salts on chloride and water absorption

EXPT.	SOLUTION	ISO-TONICITY	VOLUME		NaCl		Cl ABSORBED
			Original	Final	Original	Final	
			cc.	*cc.*	*per cent*	*per cent*	*per cent*
1	Control*	1.00	20.0	20.5	0.45	0.40	10
	2 per cent commercial bile salts (CBS)	1.09	20.0	21.2	0.44	0.46	−11
2	Control	1.00	20.0	13.0	0.45	0.24	65
	2 per cent CBS*	1.09	20.0	17.0	0.44	0.45	13
3	Control*	1.00	20.0	13.3	0.45	0.23	67
	1 per cent CBS	1.05	20.0	13.3	0.44	0.36	45
4	Control	1.00	20.0	16.3	0.45	0.36	35
	1 per cent CBS*	1.05	20.0	15.0	0.44	0.44	24
5	Control*	1.00	20.0	14.8	0.45	0.30	51
	0.2 per cent CBS	1.01	20.0	16.0	0.46	0.33	41
6	Control	1.00	20.0	14.0	0.45	0.33	48
	0.2 per cent CBS*	1.01	20.0	14.2	0.46	0.32	51

* In lower loop.

TABLE 2

Influence of sodium taurocholate on chloride and water absorption

EXPT.	SOLUTION	ISO-TONICITY	VOLUME		NaCl		Cl ABSORBED
			Original	Final	Original	Final	
			cc.	*cc.*	*per cent*	*per cent*	*per cent*
7	Control*	1.00	20.0	16.0	0.45	0.39	30
	2 per cent Na-taurocholate	1.21	20.0	19.5	0.47	0.50	−5
8	Control	1.00	20.0	15.8	0.45	0.27	52
	2 per cent Na-taurocholate*	1.21	20.0	18.5	0.47	0.55	−9
9	Control*	1.00	20.0	14.8	0.45	0.31	49
	1.5 per cent Na-taurocholate	1.00	20.0	16.3	0.41	0.40	20
10	Control	1.00	20.0	15.8	0.45	0.35	39
	1.5 per cent Na-taurocholate*	1.00	20.0	18.0	0.41	0.52	−13
11	Control*	1.00	20.0	15.0	0.45	0.26	56
	1 per cent Na-taurocholate	1.09	20.0	15.0	0.45	0.31	48
12	Control	1.00	20.0	12.0	0.45	0.28	62
	1 per cent Na-taurocholate*	1.09	20.0	12.5	0.45	0.27	63
13	Control*	1.00	20.0	13.0	0.45	0.23	67
	0.5 per cent Na-taurocholate	1.05	20.0	12.7	0.45	0.21	70
14	Control	1.00	20.0	13.3	0.45	0.24	64
	0.5 per cent Na-taurocholate*	1.05	20.0	13.5	0.45	0.29	56

* In lower loop.

carbonate and saponin. The methods used were identical with those just described.

RESULTS. The experiments with bile salts are described in tables 1 to 4. Two per cent commercial bile salts decreased water and active chloride absorption. The one per cent solution apparently decreased chloride absorption

TABLE 3

Influence of sodium glycocholate on chloride and water absorption

EXPT.	SOLUTION	ISO-TONICITY	VOLUME		NaCl		Cl ABSORBED
			Original	Final	Original	Final	
			cc.	*cc.*	*per cent*	*per cent*	*per cent*
15	Control*	1.00	20.0	15.8	0.45	0.31	46
	2 per cent Na-glycocholate	1.09	20.0	21.5	0.40	0.56	−50
16	Control	1.00	20.0	14.3	0.45	0.24	62
	2 per cent Na-glycocholate*	1.09	20.0	18.4	0.40	0.49	−13
17	Control*	1.00	20.0	15.0	0.45	0.36	40
	1.5 per cent Na-glycocholate	1.00	20.0	17.3	0.37	0.47	−9
18	Control	1.00	20.0	15.0	0.45	0.36	40
	1.5 per cent Na-glycocholate*	1.00	20.0	17.0	0.37	0.48	−8
19	Control*	1.00	20.0	12.5	0.45	0.20	72
	0.8 per cent Na-glycocholate	1.00	20.0	18.0	0.42	0.52	−13
20	Control	1.00	20.0	17.5	0.45	0.32	39
	0.4 per cent Na-glycocholate*	1.00	20.0	18.0	0.43	0.41	14
21	Control*	1.00	20.0	12.3	0.45	0.24	67
	0.4 per cent Na-glycocholate	1.03	20.0	21.5	0.46	0.40	7
22	Control*	1.60	20.0	16.3	0.45	0.37	32
	0.4 per cent Na-glycocholate	1.05	20.0	17.8	0.48	0.40	26
23	Control	1.00	20.0	13.7	0.45	0.35	47
	0.2 per cent Na-glycocholate*	1.01	20.0	16.3	0.46	0.37	35
24	Control*	1.00	20.0	14.3	0.45	0.25	61
	0.2 per cent Na-glycocholate	1.02	20.0	17.5	0.46	0.32	40

* In lower loop.

slightly but had no significant effect on water absorption. One and five-tenths per cent sodium taurocholate definitely reduced chloride and water absorption, but concentrations of 1.0 per cent or less had no influence on either process. The absorption of chloride and water was decreased by sodium glycocholate concentrations of 0.2 per cent or higher. Sodium deoxycholate decreased both types of absorption in concentrations as low as 0.2 per cent.

The results obtained with bile salt solutions cannot be explained by reduction

of chloride or sulfate concentrations, for in experiments 36 and 37, table 5, in which sodium acetate or glucose was substituted for bile salts, chloride and sulfate concentrations were considerably reduced with no significant decrease in chloride or water absorption.

TABLE 4

Influence of sodium deoxycholate on chloride and water absorption

EXPT.	SOLUTION	ISO-TONICITY	VOLUME		NaCl		Cl ABSORBED
			Original	Final	Original	Final	
			cc.	*cc.*	*per cent*	*per cent*	*per cent*
25	Control*	1.00	20.0	13.0	0.45	0.27	61
	2 per cent Na-deoxycholate	1.00	20.0	19.5	0.33	0.47	−39
26	Control	1.00	20.0		0.45		62
	2 per cent Na-deoxycholate*	1.00	20.0		0.33		−80
27	Control*	1.00	20.0	15.5	0.45	0.31	46
	1 per cent Na-deoxycholate	1.00	20.0	21.3	0.39	0.51	−40
28	Control*	1.00	20.0	14.0	0.45	0.28	56
	0.5 per cent Na-deoxycholate	1.00	20.0	17.5	0.42	0.45	5
29	Control	1.00	20.0	20.0	0.45	0.40	11
	0.5 per cent Na-deoxycholate*	1.00	20.0	21.0	0.42	0.55	−38
30	Control*	1.00	20.0	13.3	0.45	0.26	62
	0.3 per cent Na-deoxycholate	1.00	20.0	16.7	0.43	0.49	5
31	Control	1.00	20.0	13.3	0.45	0.32	52
	0.3 per cent Na-deoxycholate*	1.00	20.0	17.0	0.43	0.50	1
32	Control*	1.00	20.0	15.5	0.45	0.32	45
	0.2 per cent Na-deoxycholate	1.00	20.0	18.8	0.44	0.55	−17
33	Control	1.00	20.0	14.3	0.45	0.28	55
	0.2 per cent Na-deoxycholate*	1.00	20.0	16.3	0.44	0.42	23
34	Control*	1.00	20.0	13.5	0.45	0.26	60
	0.1 per cent Na-deoxycholate	1.00	20.0	12.2	0.44	0.27	63
35	Control	1.00	20.0	13.3	0.45	0.30	56
	0.1 per cent Na-deoxycholate*	1.00	20.0	13.8	0.44	0.38	40

* In lower loop.

Osmotic effects were probably of no importance except in experiments 7 and 8. This seems to be satisfactorily demonstrated by experiments 11 and 12, in which slightly hypertonic solutions produced no appreciable change.

The bile salts, especially sodium glycocholate and sodium deoxycholate, tend to make the solutions alkaline because they are salts of a strong base and weak

acids. The work of Ingraham and Visscher (3) suggests that this may lower the rate of active chloride absorption, but experiments 36, 38 and 39 (table 5) indicate that neither pH nor buffering power can account for the effects of bile salts obtained here. From a consideration of ionization constants (6) and molar concentrations it can be shown that the sodium acetate solution of experiment 36 must have had a higher pH and greater buffering power than the sodium glycocholate solutions having concentrations of 0.8 per cent or less. Yet the sodium acetate solution certainly did not decrease either chloride or water absorption. Similarly it can be shown that in pure water 0.042 per cent sodium bicarbonate has the same pH (9.0) as 0.2 per cent sodium deoxycholate and a higher pH than 0.2 per cent sodium glycocholate. However no significant influence of 0.042 per

TABLE 5

EXPT.	SOLUTION	ISO-TONICITY	VOLUME		NaCl		Cl ABSORBED
			Original	Final	Original	Final	
			cc.	*cc.*	*per cent*	*per cent*	*per cent*
36	Control*	1.00	20.0	15.0	0.45	0.29	52
	0.19 per cent $NaC_2H_3O_2$	1.00	20.0	13.0	0.39	0.28	52
37	Control	1.00	20.0	14.3	0.45	0.36	43
	0.75 per cent glucose*	1.00	20.0	13.5	0.39	0.34	40
38	Control*	1.00	20.0	13.0	0.45	0.30	57
	0.042 per cent $NaHCO_3$	1.00	20.0	14.0	0.44	0.27	57
39	Control	1.00	20.0	13.0	0.45	0.29	58
	0.042 per cent $NaHCO_3$*	1.00	20.0	13.5	0.44	0.27	59
40	Control*	1.00	20.0	13.0	0.45	0.26	62
	0.3 per cent saponin	1.0	20.0	17.0	0.45	0.42	20
41	Control	1.00	20.0	15.3	0.45	0.32	46
	0.15 per cent saponin*	1.0	20.0	18.0	0.45	0.47	6

* In lower loop.

cent sodium bicarbonate in an isotonic chloride-sulfate solution was demonstrated in experiments 38 and 39. Commercial bile salts and sodium taurocholate do not appreciably raise the pH of the control solution.

In two experiments saponin decreased chloride and water absorption (expts. 40 and 41, table 5).

Discussion. It should be understood that the methods used do not measure the absorptive process itself but measure net absorption only. Some of the processes which might be modified by the action of bile salts are: secretion or diffusion of chloride, secretion of water, and actual absorption of chloride or water.

In view of the fact that both bile salts and saponin lower surface tension it is possible that their mechanisms of action are similar.

Our knowledge of the bile salt concentrations which can occur in the lower ileum, either normally or in hypermotility, is not yet satisfactory. It is probable that concentrations of the order of magnitude indicated by the present experiments would interfere with salt and water absorption and tend to produce diarrhea.

The author has been unable to find in the literature any reference to effects of bile salts on chloride absorption. It is of interest to note however that Buglia (7) has reported that 0.25 per cent sodium taurocholate decreases water absorption from 0.9 per cent sodium chloride placed in the jejunum in dogs.

SUMMARY

The following concentrations of bile salts decreased water and active chloride absorption in anesthetized dogs: 2 per cent commercial bile salts, 1.5 per cent sodium taurocholate, 0.2 per cent sodium glycocholate, and 0.2 per cent sodium deoxycholate.

In general higher concentrations than these produced greater effects. The net transfer of chloride or water was toward the intestinal lumen in some cases.

Nineteen hundredths per cent sodium acetate, 0.75 per cent glucose, and 0.042 per cent sodium bicarbonate did not significantly decrease water or active chloride absorption.

The results obtained with bile salts cannot be accounted for by reduction of original chloride and sulfate concentrations, osmotic effects, pH, or buffering power.

Fifteen hundredths per cent saponin decreased water and active chloride absorption.

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THE EFFECT OF LOW POTASSIUM DIET AND OF DESOXYCORTICOSTERONE ACETATE UPON RENAL SIZE[1]

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During the course of experiments on the survival of nephrectomized rats previously fed a diet low in potassium, it was observed that this diet caused renal hypertrophy. Similar changes followed the administration of desoxycorticosterone acetate.[2] The following data are presented to elucidate the nature of these phenomena.

REVIEW OF LITERATURE. While studying the effects of potassium deficiency on tumor-bearing mice, Liebow, McFarland and Tennant (1) observed increase in kidney weight associated with tubular dilatation, hypertrophy and epithelial proliferation. They also were able to show that these changes could be reversed upon re-alimentation of the animals. Winters, Smith and Mendel (2) noted a similar increase in renal size of rats fed a salt-free diet. Kidney enlargement in rats occurred following prolonged administration of desoxycorticosterone acetate, according to Selye (3); a finding confirmed by many (4) (5). Similar renal changes have also been produced in rats and mice following injections of steroids of the ovary and testis (6) (7) (8).

METHODS. Adult albino, male and female rats, of approximately 200 grams, were used in all experiments. A control group was fed on Purina Dog Chow. The basic ingredients of the low potassium diet were present in the following proportions (9): commercial dextrin (yellow corn), 32 grams; sucrose, 25 grams; vegetable fat (Crisco), 22 grams; commercial lactalbumin (Borden's), 18 grams; dried brewer's yeast (Harris), 2 grams; bone ash, 2 grams; cod liver oil, 1 gram; and sodium chloride, 1 gram. One group of rats was fed the above diet plus 1 gram of KCl (group 3). The potassium content of the stock diet (Purina Dog Chow) was 15.5, m M/100 grams, that of the low potassium diet 1.6 m M/100 gram, and that of the synthetic diet with added potassium chloride 16.6 m M/100 grams. All animals had free access to distilled water with the exception of groups 6 and 7, to whom unlimited quantities of a 1.5 per cent solution of KCl was offered.

Crystalline desoxycorticosterone acetate was dissolved in warm alcohol and added to physiological saline so that each cubic centimeter contained 2 mgm. of finely suspended precipitate and 7 per cent alcohol. Daily subcutaneous injections of 2 mgm. of desoxycorticosterone acetate were given to groups 4, 5 and 6 during a period of four weeks.

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[2] The crystalline desoxycorticosterone acetate, percorten, used in this study was supplied through the courtesy of the Ciba Pharmaceutical Products, Inc., Summit, N. J.

The animals were sacrificed following etherization and fresh kidney weights determined. To secure dry weights the organs were placed in an oven at 100°C. The latter determination was not made in all instances as it was deemed essential to have fresh material for histological study. In these cases, the dried weights of the two kidneys were assumed to be proportionate to that of the single kidney.

RESULTS. The tabulated results of experiments are presented in table 1.

Effect of low potassium diet. Significant increase in the kidney weights of rats fed a potassium deficient diet (group 2) occurred when the results were compared to the control groups fed either a similar diet with added potassium (group 3) or stock diet (group 1). The increase in the dry weights was of the same order of magnitude. Histologically the changes were confined to the mid-medullary zone, where dilatation of the tubules with marked hyperplasia of the lining epithelium occurred, as indicated by columnar cells with numerous mitotic

TABLE 1

The effect of diet and dexoxycorticosterone acetate on kidney size

GROUP	DIET	INJECTIONS*	DRINKING FLUID	NUMBER OF RATS	MEAN BODY WEIGHT	KIDNEY WEIGHT PER KILO	t†	DRY KIDNEY WEIGHT PER KILO	t†
					grams	*grams*		*grams*	
1	Stock	0	H_2O	10	201	7.62 ± 0.41	0.00	1.77 ± 0.09	0.00
2	Low K	0	H_2O	6	248	10.53 ± 0.15	16.80	2.61 ± 0.12	3.90
3	Normal K	0	H_2O	5	257	7.37 ± 0.60	0.96	1.69 ± 0.09	1.43
4	Low K	Doca	H_2O	4	151	10.83 ± 0.81	10.00	2.43 ± 0.17	9.44
5	Stock	Doca	H_2O	18	222	9.07 ± 0.68	5.10	2.14 ± 0.14	6.79
6	Stock	Doca	1.5% KCl	5	190	7.37 ± 0.34	1.18	1.75 ± 0.08	0.40
7	Stock	0	1.5% KCl	5	204	7.22 ± 0.44	1.81	1.68 ± 0.38	1.84

* Doca indicates 2 mgm. of desoxycorticosterone acetate per day for 28 days.

† t according to Fisher (10). Value of t greater than 3.0 indicates significant difference at $p < 0.01$.

figures (figs. 1 and 2). The portions of tubule involved included the ascending loop of Henle and the collecting tubules.

Effect of desoxycorticosterone acetate. The prolonged administration of large doses of desoxycorticosterone acetate produced enlargement of the kidneys, as evidenced by the increase of both wet and dry weights. These changes were not as marked as those found in rats fed the low potassium diet. Similarly, the histological changes, while of the same nature, were not as pronounced.

Effect of low potassium diet plus desoxycorticosterone acetate. The changes in animals on a low potassium diet and injected with large quantities of desoxycorticosterone acetate were similar to those observed in the animals receiving the diet alone, and were only slightly more marked. Histologically, the changes in the two groups could not be differentiated.

Effect of desoyxcorticosterone acetate plus high potassium. The animals that received large doses of desoxycorticosterone acetate over a long period of time, supplemented by a 1.5 per cent solution of potassium chloride as the only source

of fluid, manifested no increase in renal size or changes in the concentration of water in the kidneys. No gross or microscopic alterations were evident in any of the tissues of these animals.

Effect of high potassium. Animals receiving a stock diet (Purina Dog Chow) plus a 1.5 per cent solution of potassium chloride as drinking water showed no changes in kidney size and no gross or histological changes in the tissues.

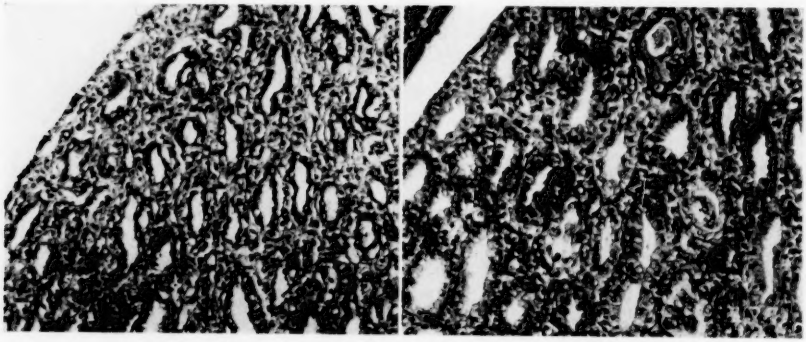

Fig. 1 Fig. 2

Fig. 1. Group 1, stock diet. Pyramid of kidney. × 130
Fig. 2. Group 2, low K diet. Pyramid of kidney. × 130

Discussion. Enlargement of the kidneys has been demonstrated previously in mice fed a diet low in potassium (1) and in rats receiving injections of desoxycorticosterone acetate. The present paper emphasizes the close relation of the two phenomena. Diets low in potassium lead to loss of muscle potassium and gain in muscle sodium (11) (12). Furthermore, injections of desoxycorticosterone acetate produce similar changes in the muscle (13) (14). Both conditions are accompanied by abnormally low serum potassium. Injections of adrenal cortical substances, as well as steroids of ovary and testis, produce increased urinary excretion of potassium (15) (16). Apparently the kidney is unable to prevent loss of potassium when animals are fed a diet low in potassium or when they receive injections of desoxycorticosterone acetate. It seems likely that the tubular hypertrophy found in these two conditions is connected with an attempt of the renal tubules to reabsorb potassium from glomerular filtrate. In the case of the low potassium diet, realimentation shows that normal ability to reabsorb potassium persists (1); on the other hand desoxycorticosterone acetate interferes with the ability to reabsorb potassium in spite of normal potassium intake. In both cases a similar tubular hypertrophy results.

The rôle of potassium is indicated by the prevention of hypertrophy when KCl supplements the low potassium diet or is added to the drinking water in rats given desoxycorticosterone acetate. These facts indicate that the tubular hypertrophy after injection of desoxycorticosterone acetate should not be regarded as a form of growth produced by hormonal stimulus but rather as hypertrophy associated with the attempt to reabsorb potassium in the presence of a deficiency.

SUMMARY

Hypertrophy of the kidney results in the rat after four weeks of a low potassium diet. The increased weight of this organ is accompanied by no significant proportionate change in the water content. The anatomical alterations are confined to the loops of Henle and collecting tubules and consist of dilatation, hypertrophy and hyperplasia.

Similar, though less marked, renal changes occur following the administration of desoxycorticosterone acetate to rats on a normal diet. These alterations are prevented by the addition of KCl to the drinking water.

Animals subjected to a high potassium regime alone show no anatomical changes.

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